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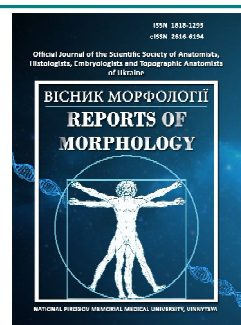
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Morphological changes in the heart of rats after serial intravesical administration of Doxorubicin

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Along with a pronounced antitumor effect, Doxorubicin causes systemic effects with damage to vital organs, including the heart. It prompts the search for ways to prevent the cardiotoxic effect of the drug, one of which could be its intravesical administration. The aim of the study was to develop a method of serial intravesical administration of Doxorubicin in medium therapeutic doses in an experiment and to evaluate the cardiotoxic effect of the drug. 42 female Wistar rats were included in the study. The control group consisted of 7 intact rats. The experimental group consisted of 35 rats who received intravesical chemotherapy with Doxorubicin at a dose of 5 mg/kg once a week for 5 weeks. On days 7th, 14th, 21st, 28th, 35th the hearts of experimental animals were taken for morphological examination. Histomorphometrically determined: the diameter of cardiomyocytes (in the middle part) and the transverse diameter of their nucleus, the width of the interstitial space (endo- and perimysium). The data of histomorphological and histomorphometric examination of the myocardium testified that all animals of the experimental group had a circulatory disorder in the heart muscle at the level of hemomicrocirculation. Such changes led to cardiomyocyte hypotrophy, interstitial edema and fibrosis. During intravesical chemotherapy, the animals showed marked changes in the myocardium, such as expansion of the endomysial zone, due to capillary congestion and edema, in comparison with animals of the intact group. At the end of the experiment, the animals of the experimental group retained the expansion of the endomysial zone, mainly due to interstitial fibrosis. Such changes indicate myocardial hypoxemia with damage and death of cardiomyocytes, activation of interstitial and replacement collagen formation. The obtained morphological data partially indicate the development of dilated cardiomyopathy in experimental animals. However, these changes were less pronounced than the previously described changes that occur after systemic administration of the drug. Additional studies of the electrophysiological activity of the heart and biochemical markers will make it possible to fully assess the degree of cardiotoxicity of Doxorubicin after its intravesical administration. Thus, serial intravesical administration of Doxorubicin in moderate therapeutic doses according to the proposed method causes changes in the myocardium of experimental animals, which are partially similar to the changes in the heart of people receiving chemotherapy with this drug.

Keywords: Doxorubicin, cardiomyopathy, morphology, experiment, rats, intravesical.

Introduction

Due to the tendency to a constant increase in overall morbidity and mortality, bladder cancer remains an urgent problem of modern medicine in general and oncology in particular [10, 16].

Bladder cancer ranks fourth in the structure of oncopathology in the United States and Europe. In terms of mortality, this pathology ranks seventh and eighth in the

structure of mortality from tumor diseases in the United States [18] and Europe, respectively [4]. Men suffer from bladder cancer about 3-4 times more often than women. According to statistics, every 26th man will develop bladder cancer during his lifetime [18]. At the same time, the overall five-year survival rate in Europe is about 68% [2].

Due to the constant improvement of diagnostic

algorithms, more and more newly diagnosed cases (up to 75% according to some data) are superficial bladder cancer, when the tumor affects only the mucous membrane or submucosal layer [1].

Transurethral resection of the tumor is a common standard in the treatment of patients with superficial bladder cancer [12, 22]. Intravesical chemotherapy and/or immunotherapy are used as adjuvant therapy after transurethral resection to prevent the development of residual and recurrent neoplasms [17, 25].

The intravesical route of drug administration opens wide opportunities for scientists and practitioners to develop new and optimize existing treatments for non-invasive bladder cancer [17, 25]. One such optimization method is intravesical administration of Doxorubicin, which has proven itself in systemic administration [5], although it has led to the development of Doxorubicin-induced cardiomyopathy [7, 9, 15, 21].

Given the morphological features of the structure of the mucous membrane of the bladder, the absorption of the chemotherapy into the systemic bloodstream, and accordingly its systemic effects should be minimal [8, 20].

However, this problem requires large-scale studies, including a preclinical study of the cardiotoxicity of Doxorubicin by intravesical administration.

The aim of the study was to develop a method of serial intravesical administration of Doxorubicin in moderate therapeutic doses in the experiment and to evaluate the cardiotoxic effect of the drug.

Materials and methods

The experimental study was performed on the basis of a research laboratory of preclinical study of pharmacological substances of National Pirogov Memorial Medical University, Vinnytsya.

All experiments were performed in accordance with the "Regulations on the use of animals in biomedical experiments" with the permission of the Bioethics Committee and in accordance with the provisions of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 "On the protection of animals used for scientific purposes".

The study included 42 female Wistar rats under 1 year of age and weighing 120.0 to 220.0 grams (192.0 ± 15.2 grams).

The control group consisted of 7 intact rats, which were selected to determine the main studied morphological and morphometric parameters in the norm. The experimental group consisted of 35 rats who underwent intravesical chemotherapy with Doxorubicin according to the author's method (Patent of Ukraine for utility model № 138090 from 25.11.2019).

The technique consisted of intravesical administration of Doxorubicin at a dose of 5 mg/kg once a week for 5 weeks. The dose of 5 mg/kg was determined by recalculating the average therapeutic dose of the drug for humans in the treatment of superficial bladder cancer. The recalculation

was performed according to the method proposed by A.B. Nair and Sh. Jacob (2016) [13].

Direct administration of the drug into the bladder was performed according to the author's method - a method of serial intravesical administration of drugs in female rats (Patent of Ukraine for utility model № 138092 from 25.11.2019) using a special catheter (Patent of Ukraine for utility model № 139761 from 27.01.2020). The catheter is a flexible tube with a diameter of 2 Fr (0.67 mm) and a length of 20 mm, at one end of which is an oil with a diameter of 6 Fr (2.0 mm), which does not cover the lumen of the catheter. The catheter was inserted into the bladder and drugs were administered as follows. Under general anesthesia, a lower-middle laparotomy was performed in experimental rats. The body of the bladder was removed into the surgical wound. A longitudinal cystotomy 3 mm long was performed. Antegradely conducted the tubular part of the catheter through the inner opening of the urethra through the urethra to the outside. The bladder was sutured. Performed revision of the abdominal cavity and washing it with an antiseptic solution. The laparotomy wound was sutured tightly in layers.

To prevent the cytostatic effect of Doxorubicin on the wound surface, its administration was started one week after catheter placement, when there was a complete restoration of the integrity of the bladder wall. To administer Doxorubicin, the tubular part of the catheter located in the outer opening of the urethra was fixed with tweezers. The syringe needle was inserted into the catheter hole and the drug was injected into the bladder.

One week after each administration of the drug (7, 14, 21, 28, 35 days), 7 rats were randomly selected and removed from the experiment by dislocation of the cervical vertebrae under ketamine anesthesia at the rate of 0.22 ml per 100 grams of weight of the experimental animal.

After dissection, the heart was removed, followed by fixation in 10% neutral formalin solution. After fixation for 3 days, cardiac samples were prepared according to standard methods. Paraffin sections 5-7 μ m thick were stained with hematoxylin and eosin. Microscopy and photographing of histological specimens were performed using a light microscope OLIMPUS BX 41 at magnifications of 40, 100, 200, 400 and 1000. Microscopy assessed the condition of the myocardium, the presence and nature of pathological and compensatory-adaptive changes in it.

Image acquisition and processing, as well as morphometry were performed using the program "Quick PHOTO MICRO 2.3". Histomorphometrically determined: the diameter of cardiomyocytes (in their middle part) and the transverse diameter of their nucleus, the width of the interstitial space (endo- and perimysia).

The obtained data were processed using the statistical software package SPSS 20.0 for Windows.

Results

The results of microscopic examination of the myocardium of the control group of animals are described

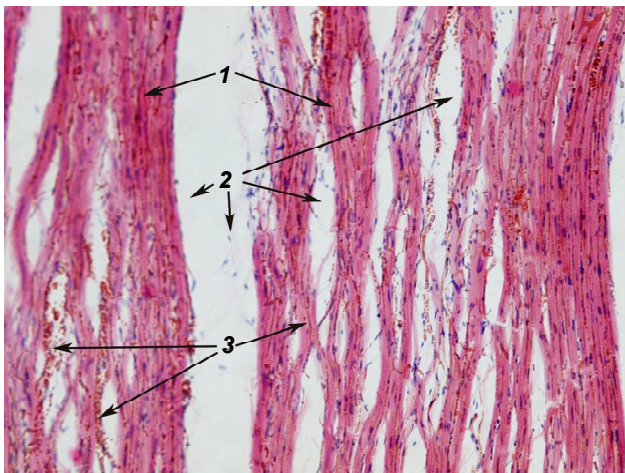


Fig. 1. The myocardium of the experimental group rat on day 7 after intravesical chemotherapy. Hematoxylin-eosin. x200. 1 - cardiomyocytes; 2 - edema of the interstitium; 3 - dilated and full-blooded vessels of hemomicrocirculation.

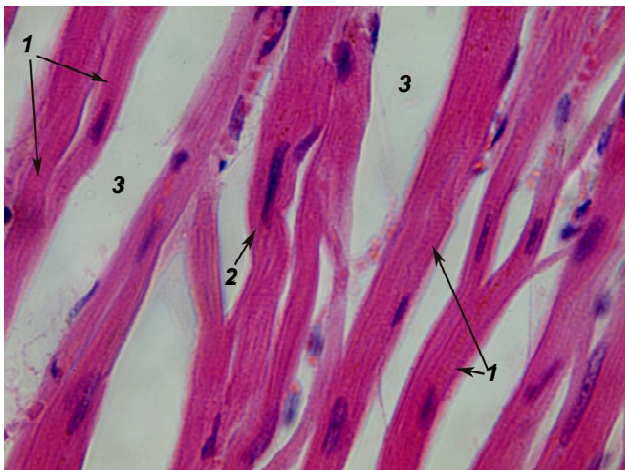


Fig. 2. The myocardium of the experimental group rat on day 7 after intravesical chemotherapy. Hematoxylin-eosin. x1000. 1 - cardiomyocytes with a clear striation; 2 - wavy tortuous cardiomyocyte; 3 - endomysium edema.

by us in a previous work [7].

Microscopically on the 7 day of the experiment in the group of animals treated with intravesical chemotherapy, the most typical were unevenly expressed disorders of hemomicrocirculation in the myocardium. Namely: capillaries and postcapillary venules had unevenly expanded lumen, signs of plethora, erythrostatics and erythrocyte sludge (in capillaries). Uneven edema of the myocardial stroma was detected. The width of the peri- and endomysium was $29.20 \pm 0.30 \mu\text{m}$ and $5.710 \pm 0.130 \mu\text{m}$, respectively (Fig. 1). The average diameter of cardiomyocytes was $11.04 \pm 0.14 \mu\text{m}$. In general, cardiomyocytes had a normal structure, a pronounced transverse striation. There were only single muscle fibers with eosinophilic homogenization and vacuolation of the sarcoplasm, basophilia and nuclear pyknosis, or enlightened sarcoplasm. These changes were mainly observed in the muscle fibers of the subendocardial

zone (Fig. 2). The transverse diameter of the nuclei averaged $3.520 \pm 0.170 \mu\text{m}$.

Histomorphologically on the 14 day of the experiment in the group of animals treated with intravesical chemotherapy, there were signs of uneven swelling of the myocardial stroma (mainly endomysia). Thus, the width of the peri- and endomysium was $31.80 \pm 0.40 \mu\text{m}$ and $5.920 \pm 0.110 \mu\text{m}$, respectively. In the vessels of hemomicrocirculation there was an uneven blood supply - there were areas with moderate capillary-venular hyperemia (sometimes with signs of erythrostatics) with anemic vessels. The average diameter of cardiomyocytes was $10.83 \pm 0.15 \mu\text{m}$, the diameter of the cross section of the nuclei - $3.420 \pm 0.130 \mu\text{m}$. In general, cardiomyocytes had a normal structure, a pronounced transverse striation, in areas with severe endomysium edema clearly manifested between them anastomoses. However, there were both single muscle fibers and small groups with eosinophilic homogenization (or uneven enlightenment) and vacuolation of sarcoplasm, basophilia, and nuclear pyknosis, not clearly visualized striation. These changes were observed mainly in the muscle fibers of the subendocardial zone and in areas with anemic vessels of hemomicrocirculation (Fig. 3).

At the histological level on the 21 day of the experiment in the myocardium of animals undergoing intravesical chemotherapy, similar to the previous period of the experiment there were dyscirculatory changes in the form of uneven interstitial edema (peri- and endomysial width averaged $30.97 \pm 0.51 \mu\text{m}$ and $5.870 \pm 0.080 \mu\text{m}$, respectively), uneven blood supply to the vessels of hemomicrocirculation and severe dilatation of lymphatic vessels perimysia, without signs of lymphostasis (Fig. 4). On average, cardiomyocytes had a diameter of $10.56 \pm 0.21 \mu\text{m}$, and the cross-sectional diameter of the nuclei was $3.290 \pm 0.140 \mu\text{m}$. Along with the vast majority of cardiomyocytes of normal histological

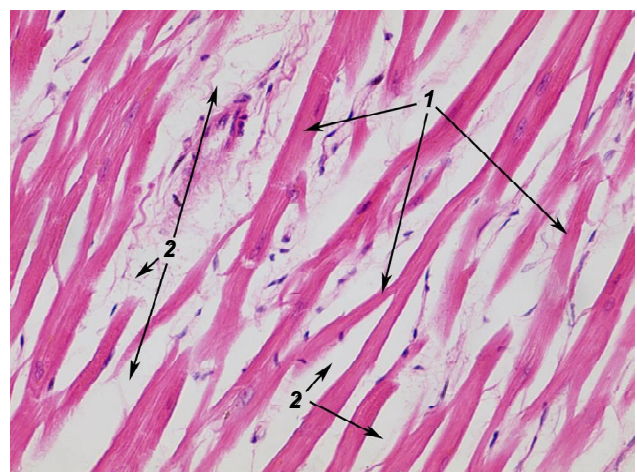


Fig. 3. The rat myocardium of the experimental group on the 14 day after intravesical chemotherapy. Hematoxylin-eosin. x400. 1 - cardiomyocytes with indistinct striation; 2 - edema of the interstitium on the background of general anemia of the microcirculatory vessels.

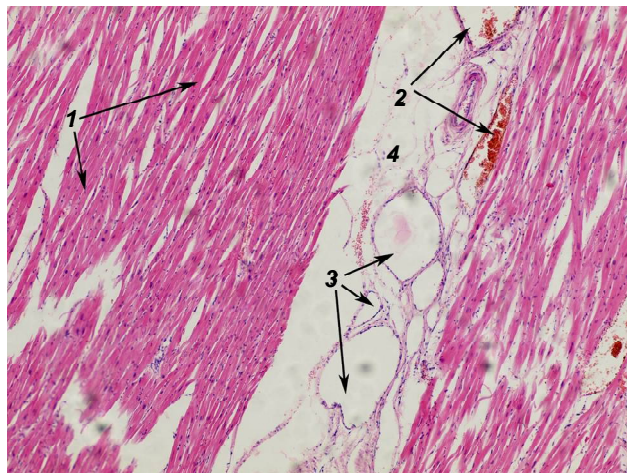


Fig. 4. The rat myocardium of the experimental group on the 21 day after intravesical chemotherapy. Hematoxylin-eosin. x100. 1 - cardiomyocytes; 2 - dilated and full-blooded vessels of hemomicrocirculation; 3 - dilated lymphatic vessels; 4 - edema of the perimysium.

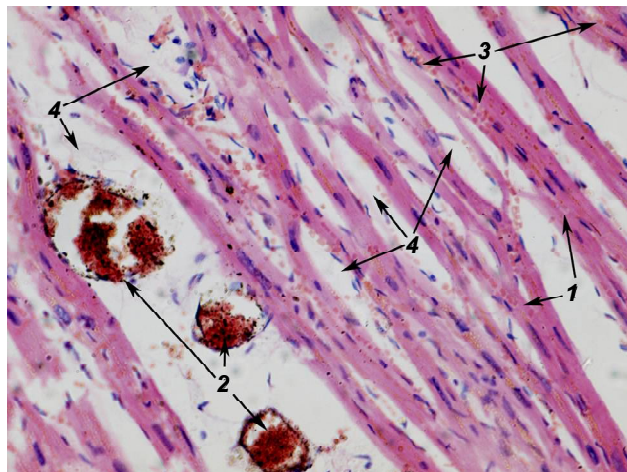


Fig. 5. The rat myocardium of the experimental group on the 28 day after intravesical chemotherapy. Hematoxylin-eosin. x400. 1 - cardiomyocytes; 2 - dilated and full-blooded venules with erythrostasis; 3 - moderate plethora of capillaries; 4 - edema of the interstitium.

structure, muscle fibers with indistinct transverse striation, violation of the tinctorial properties of sarcoplasm (eosinophilic homogenization or its enlightenment) were observed. and vacuolation of sarcoplasm, basophilia, and pyknosis of the nucleus, not clearly visualized striation.

On the 28 day of the experiment in the myocardium of animals undergoing intravesical chemotherapy, the most characteristic were hemocirculatory disorders. Namely, uneven venular (mostly) and capillary plethora, somewhere with signs of stasis, as well as moderate interstitial edema (width of the peri- and endomysium was $30.53 \pm 0.18 \mu\text{m}$ and $5.850 \pm 0.050 \mu\text{m}$, respectively). Small clusters of active fibroblasts and foci of fibrosis were found in the stroma in some places. At the same time, there were no phenomena of lymphostasis, noticeable dilation of lymphatic vessels, as in

the previous term (Fig. 5). On average, cardiomyocytes had a diameter of $10.21 \pm 0.21 \mu\text{m}$, and the cross-sectional diameter of the nuclei was $3.400 \pm 0.100 \mu\text{m}$. Among the muscle fibers of normal histological structure, there were focal fibers with indistinct transverse striation on the background of eosinophilic homogenization of sarcoplasm, uneven perception of basic stain, pyknosis or karyolysis (Fig. 6).

On the 35 day of the experiment, in the group of animals with intravesical chemotherapy, at the optical level in the myocardium there was a relatively uniform moderate blood supply to the vessels of hemomicrocirculation, local interstitial edema with dilated lymphatic vessels. The latter were observed in areas where there was excessive growth of loose unformed fibrous tissue, including endomysia. The fibrous structures of the fibrous tissue were relatively dense in places, directed mainly along the muscle fibers

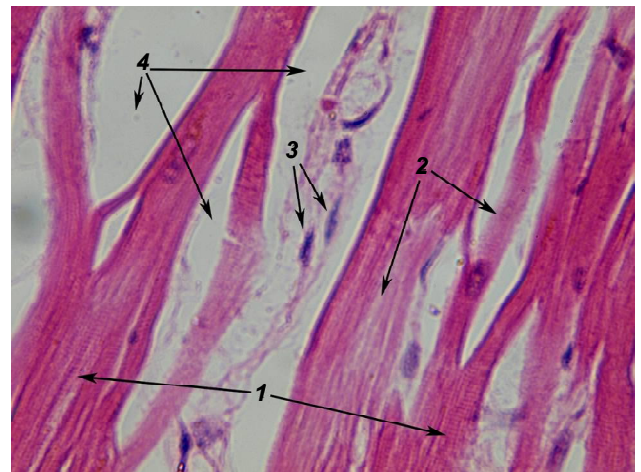


Fig. 6. The rat myocardium of the experimental group on the 28 day after intravesical chemotherapy. Hematoxylin-eosin. x1000. 1 - cardiomyocytes with a clear striation; 2 - cardiomyocytes with enlightened sarcoplasm; 3 - active interstitial fibroblasts; 4 - edema of the stroma.

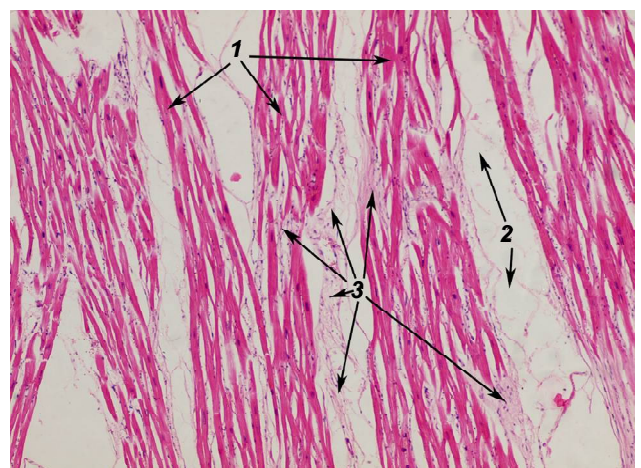


Fig. 7. The rat myocardium of the experimental group on the 35 day after the application of intravesical chemotherapy. Hematoxylin-eosin. x100. 1 - muscle fibers; 2 - edema of the stroma; 3 - interstitial focal fibrosis.

(and in places partially replaced them). Active fibroblasts, inflammatory cell elements were virtually absent. The average width of the endomysium was $5.630 \pm 0.130 \mu\text{m}$, perimysium - $30.61 \pm 0.18 \mu\text{m}$). In some cardiomyocytes their enlargement was noted, in others - compaction or enlightenment of the cytoplasm, fragmentation and defibering. Anastomoses between the fibers were moderate. The average diameter of cardiomyocytes was $10.61 \pm 0.21 \mu\text{m}$, nuclei - $3.600 \pm 0.170 \mu\text{m}$. In general, the myocardium retained its structural order. Its architecture was disturbed mainly due to focal fibrosis and edema of the interstitium (Fig. 7).

Discussion

Doxorubicin is an anthracycline drug and has been used as an antitumor agent in oncology for more than a decade [15].

Like most anticancer drugs, Doxorubicin, along with antitumor effects, has a toxic effect on vital organs, including the heart. Cardiotoxic effect is clinically manifested by the development of cardiomyopathy and congestive heart failure [9, 15, 21].

The mechanism of cardiotoxic effects of Doxorubicin has not been fully studied. However, analyzing the literature, we can identify a number of links in the pathogenesis that are key in the development of toxic heart disease. These include oxidative stress, inflammation, apoptosis, mitochondrial dysfunction, and calcium overload of cardiomyocytes [3, 11, 19, 22].

All the described pathogenetic mechanisms depend on the concentration of the drug in the blood [14, 24]. It follows that the local supply of the drug to the tumor should not only provide maximum concentration and therapeutic effect in tumor tissues, but also reduce systemic toxic effects [8, 20].

Doxorubicin is one of the drugs successfully used in the treatment of bladder cancer [5]. At superficial localization of the process there are optimal conditions for local intravesical administration of chemotherapy. The introduction of the drug into the bladder is not technically difficult. Due to the morphological features of the mucous membrane, the absorption of the drug from the bladder cavity into the blood is minimal, which should reduce its toxic effects on the body as a whole and heart tissue in particular.

In general, analyzing the data obtained by histomorphological and histomorphometric examination of the myocardium, we can conclude that all animals of the experimental group had, first of all, circulatory disorders in the heart muscle at the level of hemomicrocirculation, which,

in turn, led to damage (in mainly - dystrophy), predominant malnutrition of cardiomyocytes, interstitial edema and fibrosis. When using the intravesical method of chemotherapy, at the end of the experiment, the animals histomorphologically preserved pathological changes in the myocardium (circulatory disorders with the phenomena of stromal and intracellular edema, pathologically altered cardiomyocytes, scattered lympho-histiocytic elements and focal fibrosis in the stroma) however, the degree of their expressiveness and prevalence was insignificant. At the end of the experiment, three of the four studied indicators differed significantly from similar indicators in the control group. Thus, the diameter of cardiomyocytes was significantly smaller, and the width of the endomysium and perimysia zones was significantly larger than normal. At the same time, the diameter of the nuclei of cardiomyocytes did not differ significantly from the values in the control group.

The presence of the above changes, such as hypertrophy of some cardiomyocytes on the background of their general malnutrition, interstitial fibrosis, along with macroscopic changes in the heart, give us reason to argue about the development (under the influence of chemotherapy) in experimental animals dilated cardiomyopathy. However, the normalization of the diameter of cardiomyocyte nuclei at the end of the experiment partially indicates the normal functional activity of cardiomyocytes. In addition, changes in the diameter of cardiomyocytes and the width of the endomysium and perimysia zones were less pronounced than the previously described changes in the myocardium of rats with systemic administration of Doxorubicin [7].

Collectively, the obtained data indicate a slight cardiotoxic effect of Doxorubicin during its intravesical administration, which, in general, correlates with the literature data describing the clinical efficacy of this method of administration [6]. However, in our opinion, for a more detailed assessment of the state of the myocardium, it is necessary to further investigate the dynamics of changes in the electrophysiological activity of the heart, as well as biochemical markers of its damage.

Conclusions

1. Serial intravesical administration of Doxorubicin in moderate therapeutic doses according to the proposed method causes changes in the myocardium of experimental animals which are partly similar to changes in the heart of people undergoing chemotherapy with this drug.

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МОРФОЛОГІЧНІ ЗМІНИ У СЕРЦІ ЩУРІВ ПРИ СЕРІЙНОМУ ВНУТРІШНЬОМІХУРОВОМУ ВВЕДЕННІ ДОКСОРУБІЦИНУ

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Поряд з вираженим протипухлинним ефектом Доксорубіцин спричиняє системний вплив із ураженням життєво важливих органів, зокрема серця. Це спонукає до пошуку способів попередження кардіотоксичного впливу препарату, одним із яких може бути внутрішньоміхурове його введення. Мета дослідження - розробити спосіб серійного внутрішньоміхурового введення Доксорубіцину у середньотерапевтичних дозах в експерименті та оцінити кардіотоксичний ефект препарату. До дослідження були включені 42 самки щурів лінії Wistar. Контрольну групу склали 7 інтактних щурів. Дослідну групу склали 35 щурів, котрим проводили внутрішньоміхурову хіміотерапію Доксорубіцином у дозі 5 мг/кг 1 раз на тиждень протягом 5

тижнів. На 7, 14, 21, 28 та 35 добу вилучали серця дослідних тварин для морфологічного дослідження. Гістоморфометрично визначали: діаметр кардіоміоцитів (у серединній їх частині) і поперечний діаметр їх ядра, ширину інтерстиціального простору (ендо- та перимізія). Дані гістоморфологічного та гістоморфометричного дослідження міокарда свідчили про те, що у всіх тварин дослідної групи мало місце порушення кровообігу в серцевому м'язі на рівні гемомікроциркуляції. Такі зміни призводили до гіпотрофії кардіоміоцитів, інтерстиціального набряку та фіброзу. При проведенні внутрішньоміхурової хіміотерапії у тварин відмічалися виражені зміни міокарда, такі, як розширення зони ендомізію, за рахунок капілярного повнокрів'я та набряку, у порівнянні з тваринами інтактної групи. Наприкінці експерименту у тварин дослідної групи зберігалось розширення зони ендомізію в основному за рахунок інтерстиціального фіброзу. Подібні зміни вказують на гіпоксемію міокарда з ушкодженням та загибеллю кардіоміоцитів, активацією інтерстиціального та замісного колагеноутворення. Отримані морфологічні дані частково свідчать про розвиток у експериментальних тварин дилатаційної кардіоміопатії. Проте дані зміни були менш вираженими, ніж описані нами раніше зміни, що виникали після системного введення препарату. Додаткові дослідження електрофізіологічної діяльності серця та біохімічних маркерів дозволять повною мірою оцінити ступінь кардіотоксичності Доксорубіцину при його внутрішньоміхуровому введенні. Таким чином, серійне внутрішньоміхурове введення Доксорубіцину у середньотерапевтичних дозах за запропонованою методикою викликає в міокарді експериментальних тварин зміни, котрі частково подібні до змін у серці людей, котрі отримують хіміотерапію даним препаратом.

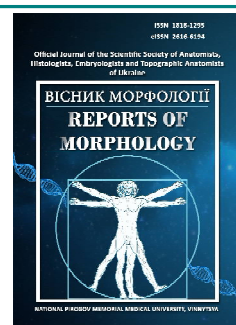
Ключові слова: доксорубіцин, кардіоміопатія, морфологія, експеримент, щури, внутрішньоміхуровий.



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Biochemical parameters of blood and urine of rats at six-week administration of Nalbuphine

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Given the widespread use in medicine of opioid analgesics, today it is extremely important to study the morphofunctional changes of various organs with prolonged exposure to opioids. In the modern scientific literature there are works in which changes of biochemical indicators of blood at use of narcotic analgesics in experiment are covered. To study the changes in the biochemical parameters of blood and urine with long-term use of Nalbuphine is an important area of research. The aim of the study was to detect changes in some biochemical parameters of blood and urine of rats at six-week administration of therapeutic doses of Nalbuphine. The study was based on 40 white adult male rats weighing 180-260 g, which were injected daily for six weeks with Nalbuphine injection 20 mg daily, intramuscularly in therapeutic doses. Daily diuresis and creatinine content were determined in urine samples. The content of total protein, creatinine, urea, calcium and phosphorus was determined in blood samples. The glomerular filtration rate was also determined by the clearance of endogenous creatinine. All statistical calculations were performed using R Studio v.1.1.442 and R Commander v.2.4-4 software. The results of the study showed that six weeks of therapeutic doses of Nalbuphine affects the filtration and reabsorption function of the kidney. The use of Nalbuphine for two weeks does not cause significant changes in biochemical parameters, but the established deviations may indicate the first signs of renal dysfunction. Further use of Nalbuphine, including the end of the sixth week of the experiment, leads to significant changes in the filtration and reabsorption functions of the kidneys, as evidenced by statistically significant changes in the biochemical parameters of blood and urine of rats. The data of the study can be used to compare with changes in the functional state of the kidneys caused by prolonged use of Nalbuphine, its withdrawal and the use of other drugs in combination with Nalbuphine. It is also advisable to compare the results of the study with the morphological changes of the kidneys at different times of use of Nalbuphine.

Keywords: kidney, opioids, biochemical parameters.

Introduction

It is known that opioids are effective drugs in the symptomatic treatment of chronic pain of various etiologies, but have a toxic effect with prolonged use [4, 17, 19]. Current issues for now are the study of morphofunctional changes in various organs under the influence of opioids [9, 14, 20]. Currently, there are works that highlight the changes in the biochemical parameters of the blood when using narcotic analgesics in the experiment [10, 13]. The morphofunctional state of the kidney under the influence of various drugs and chemical compounds is studied by many scientists [3, 24]. A number of studies are devoted to the study of changes in the structure and biochemical parameters of renal function

with the use of opioids. Morphological changes of the kidney with the use of opioids were studied both on autopsy material and in an experiment [7, 11, 27]. Intravenous use of heroin leads to nephropathy, which is characterized by a morphological picture of focal and segmental glomerulosclerosis. This nephropathy was defined as heroin-associated in the early 1970s [21] and was found to be caused by the toxic effects of substances used to chemically process "street" heroin [6, 23]. Cases of renal lipoidosis detected by renal biopsy of patients using Methadon have been described [21]. Chronic kidney damage with opioid use may occur due to the method of

drug administration, in particular intradermal drug administration to cause amyloidosis [15, 16]. Thirty-day administration of morphine and tramadol in the experiment on rats revealed vacuolation of epithelial cells of the nephron tubules, interstitial mononuclear infiltration, focal necrosis and hemorrhage [1]. Among the causes of acute kidney damage when using opioids may be renal ischemia, resulting in decreased glomerular filtration rate, hypotension, dehydration, as well as rhabdomyolysis and acute urinary retention [5, 15]. Opiate use may be a risk factor for decreased renal function and/or albuminuria [18]. A study of the relationship between the use of analgesics, including opioids, with biomarkers of chronic kidney disease in a representative sample of adults in the United States found higher rates of albuminuria when using prescription opioids compared with non-opioids [2]. Prolonged administration of Tramadol and Morphine in the experiment leads to increased creatinine levels [1].

In view of the above, the study of biochemical parameters of blood and urine with long-term administration of opioid analgesics, in particular Nalbuphine, is an important area of research.

The aim of our study was to identify changes in some biochemical parameters of blood and urine of rats with a six-week administration of Nalbuphine therapeutic doses.

Materials and methods

The studies were performed on 40 white sexually mature male rats weighing 180-260 g. The animals were kept in a vivarium on a standard diet with free access to water, constant humidity and temperature [8].

The work was carried out in accordance with the "Rules for the use of experimental animals", which corresponds to the provisions of the Declaration of Helsinki. Experimental animals were administered Nalbuphine injection 20 mg daily for six weeks, intravenously in therapeutic doses. The control group received intramuscular injections of saline for six weeks.

Marked and weighed animals were kept in metabolic cells the day before blood collection and daily diuresis and

urine creatinine were determined. Blood for the study was collected after intraperitoneal administration of sodium thiopental from the inferior vena cava and the content of total protein, creatinine, urea, calcium and phosphorus was determined. In order to assess the functional status of the kidneys, the glomerular filtration rate was determined by the clearance of endogenous creatinine [12].

The data obtained by us were checked for the normality of the distribution using the indicators of the asymmetry and excess coefficients, as well as using the Shapiro-Wilk test. All statistical calculations were performed using R Studio v. 1.1.442 and R Commander v.2.4-4.

Results

Changes in some biochemical parameters of rat blood and urine have been reported with daily intramuscular administration of Nalbuphine therapeutic doses. The data are presented in Table 1.

In the study, at 6 weeks of therapeutic doses of Nalbuphine, serum protein values tended to decrease in the second and fourth weeks of the experiment compared with the control group (65.90±4.58) g/L and amounted to (62.40±3.81) g/L and (62.40±2.50) g/L, respectively. However, these changes had no statistical confirmation.

Serum urea values after two and four weeks had no statistically significant difference compared with the control group ($p > 0.05$). After 6 weeks of the experiment, the average value of urea was statistically significantly higher ($p < 0.05$) compared with the control group and was (7.623±0.729) mmol/L with the control group indicator (5.914±0.719) mmol/L.

After two weeks of the experiment, a tendency to increase the level of creatinine in the serum (53.02±7.56) $\mu\text{mol/L}$ was found, although compared with the control value of the average value (48.35±10.83) $\mu\text{mol/L}$, this was not confirmed statistically ($p < 0.05$). In the fourth and sixth weeks of the experiment there was a significant ($p < 0.05$) increase in the value of creatinine compared with the control and the previous term, which was (78.98±4.54) and (107.9±7.4) $\mu\text{mol/L}$, respectively.

Table 1. Indicators of protein, urea creatinine, blood calcium and phosphorus, daily diuresis, urine creatinine and glomerular filtration rate of rats at 6 weeks of therapeutic doses of Nalbuphine, $M \pm SD$ ($n=10$).

Indexes	Control	2 weeks	4 weeks	6 weeks
Total protein, g/L	65,90±4,58	62,40±3,81	62,40±2,50	64,40±3,57
Urea, mmol/L	5,914±0,719	6,222±0,468	5,732±0,354	7,623±0,729 [°]
Blood creatinine, $\mu\text{mol/L}$	48,35±10,83	53,02±7,56	78,98±4,54 ^{*°}	107,9±7,4 [°]
Calcium, mmol/L	2,341±0,163	2,157±0,086 [*]	2,394±0,097 [°]	2,391±0,090 ^{**}
Phosphorus, mmol/L	2,256±0,178	2,424±0,049 [*]	2,317±0,113	2,115±0,118 [°]
Daily diuresis, ml/day	8,680±2,613	9,520±1,630	11,10±1,96 [*]	15,85±1,79 [°]
Urine creatinine, $\mu\text{mol/L}$	2535±458	2829±237	1521±138 ^{*°}	3237±130 [°]
Glomerular filtration rate, $\mu\text{l/min}$	307,0±96,1	367,4±74,0	146,6±31,2 ^{*°}	330,4±36,7 [°]

Notes: * - $p < 0,05$ compared to control; ° - $p < 0,05$ compared to the previous term of the experiment; ** - $p < 0,05$ compared with the second week of the experiment.

In the second week of the experiment, the parameters of rat blood calcium decreased to the values (2.157 ± 0.086) mmol/L, which was statistically significant ($p < 0.05$) compared with the control group (2.341 ± 0.163) mmol/L. However, later, after 4 and 6 weeks of the experiment, the calcium values increased and were statistically significantly higher ($p < 0.05$), compared with the calcium values in the second week of the experiment and were (2.394 ± 0.097) mmol/L and (2.391 ± 0.090) mmol/L, respectively. Such values did not have a statistically significant difference with the control group.

Rat blood phosphorus values in the second week significantly ($p < 0.05$) increased to (2.424 ± 0.049) mmol/L compared with the control group (2.256 ± 0.178) mmol/L. In the fourth week of the experiment, blood phosphorus values decreased slightly compared to the previous term and amounted to (2.317 ± 0.113) mmol/L. At the sixth week of the experiment, the serum phosphorus concentration continued to decrease and reached the minimum values - (2.115 ± 0.118) mmol/L, which is significantly lower ($p < 0.05$) than the previous term. It should be noted that compared with the control group, the decrease in phosphorus concentration was insignificant ($p > 0.05$).

The tendency to increase the daily diuresis was observed in the second week of the experiment (9.520 ± 1.630) ml/day, although the difference was not statistically significant compared with the control group, where the daily diuresis was (8.680 ± 2.613) ml/day. A significant increase in daily diuresis compared to the control indicator was found after 4 weeks of the experiment - (11.10 ± 1.96) ml/day. After six weeks of the experiment, the daily diuresis was (15.85 ± 1.79) ml/day and was statistically significantly higher than the control group and the previous term.

After two weeks of the experiment, there was a tendency to increase the urinary creatinine (2829 ± 237) $\mu\text{mol/L}$, although the difference was not statistically significant compared with the control group (2535 ± 458) $\mu\text{mol/L}$. After four weeks of the experiment, a significant sharp (1.67 times), compared with the control group, a decrease in urine creatinine (1521 ± 138) $\mu\text{mol/L}$ ($p < 0.05$). After six weeks of the experiment, the level of urine creatinine increased 2.13 times compared with the fourth week of the experiment and 1.28 times compared with the control group and was (3237 ± 130) $\mu\text{mol/L}$, which was statistically significant as when comparing this value in period of 4 weeks, and with similar numerical values obtained in the control group.

After two weeks of the experiment, there was a tendency to increase the glomerular filtration rate (2829 ± 237) $\mu\text{l/min}$, although the difference was not statistically significant compared with the control group (307.0 ± 96.1) $\mu\text{l/min}$. In the fourth week of the experiment, the glomerular filtration rate decreased sharply (146.6 ± 31.2) $\mu\text{l/min}$, 2.1 times compared with the control group. At the end of the sixth week of the experiment, the glomerular filtration rate increased 2.25 times

compared to the previous term, is slightly higher than the control group and is (330.4 ± 36.7) $\mu\text{mol/L}$.

Discussion

Prolonged use of opioid analgesics leads to changes in the structure and function of various systems and organs, including the kidneys [25, 26]. Given the use in medical practice, in particular in chronic pain syndrome, therapeutic doses of Nalbuphine, there is a need to study the morphofunctional changes of the kidneys with long-term use of this drug in the experiment.

As a result of our biochemical study of some indicators of blood and urine of rats under the conditions of six weeks of exposure to Nalbuphine, the following changes in the functional state of the kidney were found. The use of therapeutic doses of Nalbuphine for two weeks leads to minor changes in serum protein, urea, creatinine, calcium and phosphorus, daily diuresis and urinary creatinine, glomerular filtration rate. Such changes in indicators are not statistically significant compared with the control group, but may indicate the initial signs of changes in renal structures and the beginning of the development of filtration and reabsorption disorders, which at this time are still compensated.

Researchers data suggest that long-term use of opioids may lead to a decrease in glomerular filtration rate [18]. At the end of the fourth week of the experiment, we observed a significant decrease in glomerular filtration rate due to a 1.63-fold increase in serum creatinine combined with a sharp 1.67-fold decrease in urinary creatinine. At this time, impaired renal filtration function appears due to a sharp decrease in glomerular filtration rate. The revealed significant increase in the daily diuresis also indicates a violation of the reabsorption function of the kidneys.

The mechanisms of opioid-related renal failure are renal ischemia (prerenal factor), acute tubular necrosis, and impaired urinary outflow due to impaired bladder innervation (postrenal factor) [15]. At the end of the sixth week of the experiment, we observed progression of renal filtration function impairment, as evidenced by a 2.23-fold increase in serum creatinine and a 1.29-fold increase in serum urea relative to the control group. Such changes in the indicators of nitrogen excretory function of the kidney may indicate damage to the structure of the renal corpuscle. However, an increase in the glomerular filtration rate may indicate the accession of compensatory mechanisms in this period. The use of Nalbuphine for six weeks revealed a deepening of renal reabsorption function, as evidenced by an increase in the number of daily diuresis and a tendency to decrease serum phosphorus, and indicates damage to the convoluted tubules of the nephron.

The data of the study can be used in the future to compare with changes in the functional state of the kidneys caused by prolonged use of Nalbuphine, its withdrawal and the use of other drugs in combination with Nalbuphine. Also, the results of the study should be compared with the

morphological changes of the kidneys at different periods of Nalbuphine use.

Conclusions

1. The results of the study of therapeutic doses Nalbuphine during six weeks indicate an effect on the filtration and reabsorption function of the kidney by this drug.
2. The use of Nalbuphine for two weeks does not cause

significant changes in biochemical parameters, but the established deviations may indicate the first signs of renal dysfunction.

3. Further use of Nalbuphine, including the end of the sixth week of the experiment, leads to significant changes in the filtration and reabsorption functions of the kidneys, as evidenced by statistically significant changes in the biochemical parameters of rats' blood and urine.

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БІОХІМІЧНІ ПОКАЗНИКИ КРОВІ ТА СЕЧІ ЩУРІВ ПРИ ШЕСТИТИЖНЕВОМУ ВВЕДЕННІ НАЛБУФІНУ

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Враховуючи широке використання в медицині опіоїдних анальгетиків, сьогодні надзвичайно актуальним є вивчення морфофункціональних змін різних органів при тривалому впливі опіоїдів. У сучасній науковій літературі є роботи, в котрих висвітлені зміни біохімічних показників крові при вживанні наркотичних анальгетиків в експерименті. Дослідити зміни біохімічних показників крові та сечі за умови довготривалого використання налбуфіну є актуальним напрямком наукового дослідження. Мета дослідження - виявити зміни деяких біохімічних показників крові та сечі щурів при шеститижневому введенні терапевтичних доз налбуфіну. Матеріалом дослідження слугували 40 білих статевозрілих щурів самців масою 180-260 г, котрим впродовж шести тижнів проводили ін'єкції налбуфіну (Nalbuphine injection 20 mg) щоденно, дом'язово у терапевтичних дозах. У пробах сечі визначали добовий діурез та вміст креатиніну. У пробах крові визначали вміст загального білка, креатиніну, сечовини, кальцію та фосфору. Також визначали показник швидкості клубочкової фільтрації за кліренсом ендogenous креатиніну. Всі статистичні обрахунки проводили із використанням програмного забезпечення R Studio v.1.1.442 та R Commander v.2.4-4. Результати дослідження показали, що шеститижнєве застосування терапевтичних доз налбуфіну впливає на фільтраційну та реабсорбційну функції нирки. Використання налбуфіну впродовж двох тижнів не викликає достовірних змін біохімічних показників, проте встановлені відхилення можуть свідчити про перші ознаки порушення функцій нирок. Подальше використання налбуфіну, включно до закінчення шостого тижня експерименту, призводить до достовірних змін фільтраційної та реабсорбційної функцій нирок, про що свідчать статистично достовірні зміни біохімічних показників крові та сечі щурів. Дані проведеного дослідження можуть бути використані для порівняння зі змінами функціонального стану нирок, викликаними тривалішим застосуванням налбуфіну, його відміною та застосуванням інших медичних препаратів у поєднанні з налбуфіном. Також результати дослідження доцільно співставити з морфологічними змінами нирок на різних термінах застосування налбуфіну.

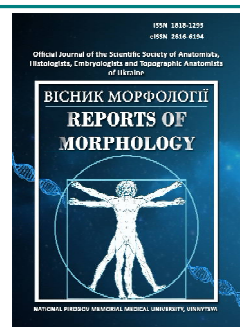
Ключові слова: нирка, опіоїди, біохімічні показники.



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Morphometric features of rat pinealocytes in conditions of chronic ethanol intoxication

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Ethanol has chronobiological effects, which are associated with inhibition of melatonin synthesis and secretion and disruption of the sleep-wake cycle. Ethanol is known to cause sleep fragmentation due to frequent awakenings, prolong wakefulness, and reduce the duration of the slow sleep phase. At the same time, changes in the morphology of the pineal gland under chronic exposure to ethanol remain poorly studied. Of particular interest are changes in the basic morphometric parameters of pinealocytes, because they are a marker of the functional state of the pineal gland. The aim of the study was to morphometrically study the features of morphological changes in rat pinealocytes in the physiological norm and in chronic ethanol intoxication. To achieve this goal, we used 20 laboratory male rats: a control group and an experimental group. The control group was under standard vivarium conditions. For the experimental group, alcohol intoxication was modeled by injecting a 40% ethanol solution at the rate of 12 mg/kg of body weight intragastric 4 times a day. The morphometric parameters of pinealocytes were studied on day 30 from the start of the study. According to the results of morphometric measurements, a significant increase in the parameters of light pinealocytes and a decrease in the parameters of dark cells were established. It was determined that the average values of the cytoplasm area of light pinealocytes increase by 54.55% ($p < 0.05$), the area of the nucleus increases by 61.32% ($p < 0.05$), and the area of the nucleolus by 32.84% ($p < 0.05$) compared with the control group. The area of the cytoplasm of dark pinealocytes decreases by 27.2% ($p < 0.05$), and the area of the nucleus by 37.33% ($p < 0.05$). Changes in the ratio of light and dark pinealocytes were also noted. An increase in the number of active light cells by 8.17% was found. The detected morphometric changes indicate high functional activity of cells, which has a compensatory nature in response to apoptosis of pinealocytes.

Key words: pineal gland, chronic ethanol intoxication, pinealocytes, vacuolar dystrophy.

Introduction

In recent years, there has been a significant increase in the number of cases of intoxication due to alcohol abuse, due to a sharp increase in the level of alcoholism, an increase in the number of low-quality alcoholic beverages. According to official statistics, the level of alcohol consumption in our country is one of the highest compared to all other countries in the world. There are reports in the literature that the level of alcohol consumption increased significantly during the COVID-19 pandemic and was due to increased stress, depression, boredom and the availability of alcoholic beverages [2, 10]. The World Health Organization (WHO) considers alcohol abuse as a global medical and social problem of the XXI century [16, 17].

It is known that ethanol and its metabolites have a toxic

effect on almost all organs and systems of the body. The greatest impact as a result of ongoing chemical reactions aimed at the excretion of ethanol and detoxification of the body are the liver and kidneys. Changes in the morphology of various organs of visceral systems are described in detail in the literature [1, 16, 17, 18, 19, 22]. At the same time, the effect of ethanol on the morphofunctional state of various parts of the brain has not been studied enough. The vast majority of publications are devoted to changes in the morphology of the cerebellum under the influence of ethanol [14, 20, 21, 24].

There is information that ethanol has chronobiological effects that are associated with inhibition of melatonin synthesis and secretion and affects the sleep-wake cycle,

in particular causes fragmentation of the cycle due to frequent awakenings, prolongs wakefulness, reduces the duration of the slow phase of sleep [3, 5, 7, 12, 23]. However, changes in the morphology of the pineal gland under the influence of ethanol remain poorly understood [15]. Of particular interest are the changes in the basic morphometric parameters of pinealocytes, because they are a marker of the functional state of the pineal gland during the experiment.

The aim of our work was to study the morphometric features of rat pinealocytes in conditions of chronic ethanol intoxication.

Materials and methods

The results of this work are a fragment of the research topic of the Department of Anatomy, Clinical Anatomy, Operative Surgery, Pathomorphology and Forensic Medicine of the Petro Mohyla Black Sea National University of MES of Ukraine "The role of environmentally hazardous factors in the development of diseases of civilization", state registration № 0120U002026.

The experimental studies involved 20 adult male Wistar rats, weighing 180-220 g. The animals were kept in standard vivarium conditions. Experimental animals were divided into 2 groups: control and experimental. The control group included 6 animals, the experimental group 14. The first group consisted of intact rats, which were under normal conditions without the influence of additional factors. The second group of animals was simulated alcohol intoxication by administering 40% ethanol solution at a rate of 12 mg/kg body weight intragastrically 4 times a day [19, 25, 26]. On day 30 after the start of the experiment, rats were euthanized with thiopental anesthesia at a rate of 25 mg/kg body weight.

In order to perform morphological studies, the pineal gland was isolated [8] and fixed in a 10% solution of neutral formalin, dehydrated in solutions of ethyl alcohol with increasing concentration (40-96°) and poured into paraffin. Sections with a thickness of 4-6 µm were made on a rotary microtome of the semi-automatic type of the "Microm" brand (Germany). For further light-optical examination, histological specimens were stained according to the conventional method with hematoxylin and eosin. Light microscopy and microphotography of histopreparations were performed using a microscope brand "Carl Zeiss" (Germany) at a magnification of objective lens x20, ocular lens x10.

The number of pinealocytes in the field of view of the microscope was counted on the histological preparations of the pineal gland obtained in this standard way. The field of view of the microscope in our studies (objective lens x20, ocular lens x10) was taken as a conventional unit of area in which the number of pineal cells was counted. Cell counting was performed by analogy with the counting chamber and used Egorov's rule, namely pinealocytes were counted in the field of view and on the border of the

upper and right sectors, and cells located on the lower and left border of the microscope field of view were not counted. To obtain statistically significant data, the number of cells in 10 fields of view of the microscope was determined.

Morphometric measurements were performed using a micrometer eyepiece screw type MOV 1-16 at magnification (x40). 10 histological preparations of the studied organ of each experimental group were analyzed. Histological preparations of the control and experimental groups were studied in parallel.

Large and small diameters of the nucleus and cytoplasm of both cell types were measured. The parameters of the nucleoli of pinealocytes were determined in one direction, which can be explained by the rounded shape of these cell structures. The calculation of the area of the nucleus and cytoplasm was performed using the formula: $S = \pi r R$, where: r is a small radius; R is a large radius. Since the nucleoli have a rounded shape, their area was determined using a well-known mathematical formula to calculate the area of structures that have the form of a circle: $S = \pi r^2$.

The determined quantitative morphometric data, which characterized the parameters of pinealocytes and their changes, were entered into the electronic journal of studies and subjected to statistical analysis using conventional methods of variation statistics, namely Student's t-test. Mathematical and statistical processing of the obtained numerical results was performed on a personal computer using the standard software "STATISTICA 6" for computer equipment with the Windows operating system. For all indicators, arithmetic mean values, standard errors of arithmetic mean and standard deviation were calculated, which are denoted in the work by the corresponding symbols $M \pm m$ and σ . The difference was considered significant when the difference of numerical parameters between the intact and experimental series at the level of not less than $p < 0.05$. This level of reliability is common in mathematical and statistical research in the biological and medical fields.

All manipulative interventions on experimental animals were carried out in strict compliance with the provisions of the 1997 Convention on Bioethics of the Council of Europe, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the general ethical principles of animal experiments. (September 2001), the Law of Ukraine "On Protection of Animals from Cruelty" (2006) and other international agreements and current national legislation in the field of medical and biological research.

Results

Microscopic examination of the pineal gland of rats revealed that the overall structure of the parenchyma of the organ is typical. The parenchyma is formed by two types of pinealocytes, which form the lobes of the organ. At the same time, remodeling of pinealocytes was revealed, which

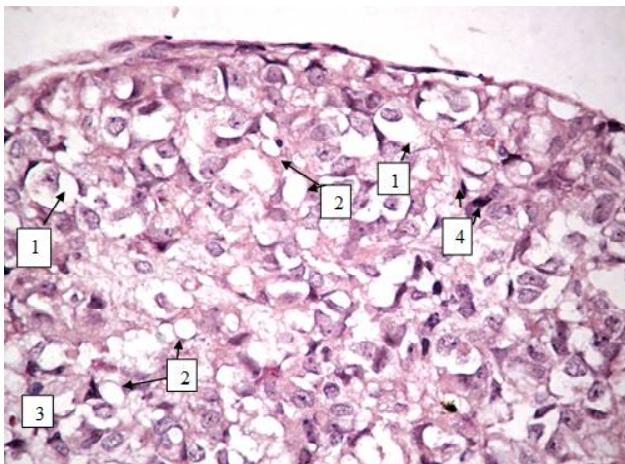


Fig. 1. A fragment of the parenchyma of the rat pineal gland under conditions of ethanol intoxication. HE. Oc. x10, ob. x20. 1 - T1P in a state of vacuolation; 2 - vacuoles filled with cytoplasmic fluid; 3 - T2P; 4 - glial cells.

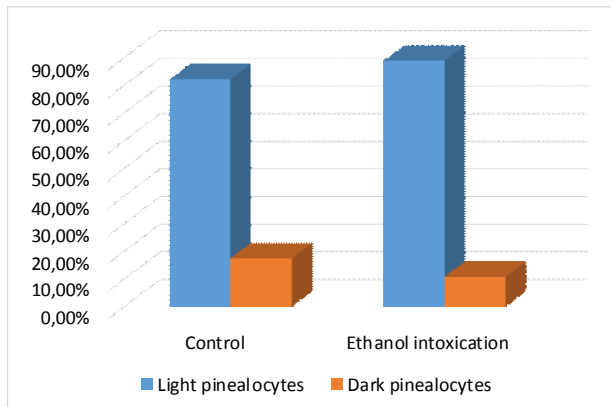


Fig. 2. Graphic ratio of T1P and T2P of the pineal gland parenchyma in experimental animals under the influence of ethanol compared with the control group of animals.

Table 1. Morphometric parameters of T1P in the control group of animals and under the influence of ethanol.

Group of animals	Cytoplasm area, μm^2 (M \pm m, n=50)	Nuclei area, μm^2 (M \pm m, n=50)	Nucleoli area, μm^2 (M \pm m, n=50)
Control	45,28 \pm 1,32	16,83 \pm 0,71	2,040 \pm 0,100
Ethanol intoxication	69,98 \pm 2,85*	27,15 \pm 1,27*	2,710 \pm 0,220*

Notes: * reliably with control ($p < 0,05$).

accordingly affected their morphometric parameters. It was found that cell remodeling was manifested by their edema, enlightenment and vacuolation of the cytoplasm of the vast majority of T1P (Type 1 pinealocytes, light pinealocytes). A large number of vacuoles of different sizes were found on histological specimens, which indicates the death of overloaded cells (Fig. 1). Due to such changes, the cells are located in the parenchyma sparsely.

It was found that the absolute majority in the histological sections of the pineal glands of both the control and experimental groups were T1P. However, it is necessary to note changes in the ratio between T2P (Type 2 pinealocytes,

dark pinealocytes) and T1P. It was determined that in the animals of the control group the percentage of T1P from the total number of pinealocytes was $82.35 \pm 0.68\%$, and the percentage of T2P was $17.65 \pm 0.73\%$. Under conditions of chronic exposure to ethanol, the number of T1P increased compared to the control group, and T2P - decreased. It was found that the percentage of T1P in the experimental group was $89.08 \pm 0.91\%$, and the percentage of T2P decreased to $10.92 \pm 1.21\%$ (Fig. 2). Such changes in the ratio of cells indicate the transformation of T2P in the T1P, which occurs due to the loss of functioning overloaded cells.

Given the fact that the number of T1P is dominant for both intact and experimental groups, a sample of 50 for T1P and 30 for T2P was chosen to determine and analyze the morphometric parameters of pinealocytes.

In the comparative analysis of the average cytoplasm, nuclei and nucleoli of T1P in rats of the control and experimental groups, probable differences were found.

It was determined that the average values of the cytoplasm area of T1P in the physiological norm were $45.28 \pm 1.32 \mu\text{m}^2$, nuclei - $16.83 \pm 0.71 \mu\text{m}^2$, nucleoli $2.040 \pm 0.100 \mu\text{m}^2$ (Table 1). On the 30 day of the study of the effects of ethanol, the average area of the cytoplasm of T1P was $69.98 \pm 2.85 \mu\text{m}^2$, which was significantly higher (54.55%) than in animals of the control group ($p < 0.05$). The area of the nucleus similarly exceeded the corresponding parameter of the control group by 61.32% ($p < 0.05$). Its average values were $27.15 \pm 1.27 \mu\text{m}^2$. The average area of the nucleolus significantly increased by 32.84% compared with the control group and was equal to $2.710 \pm 0.220 \mu\text{m}^2$ (Fig. 3). Such changes in the parameters indicate an increase in the synthetic activity of T1P.

When comparing the morphometric data of T2P of both groups, a significant decrease in the average morphometric parameters of rats exposed to ethanol intoxication was observed (Table 2).

On the 30 day of the experiment, the area of the cytoplasm of T2P decreased by 27.2% ($p < 0.05$) compared with the intact group and was $20.85 \pm 1.94 \mu\text{m}^2$. The average

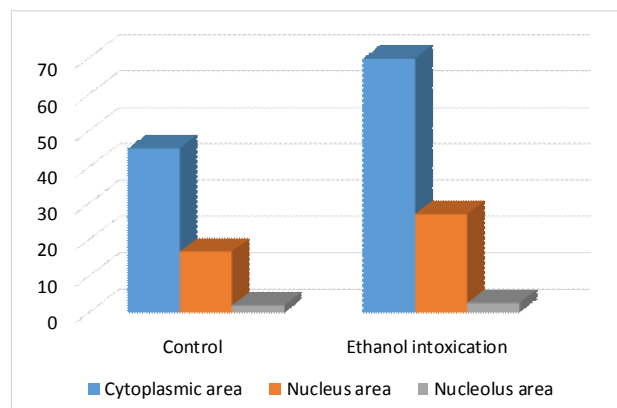


Fig. 3. Comparison of mean values of T1P morphometric parameters in control and experimental groups, μm^2 .

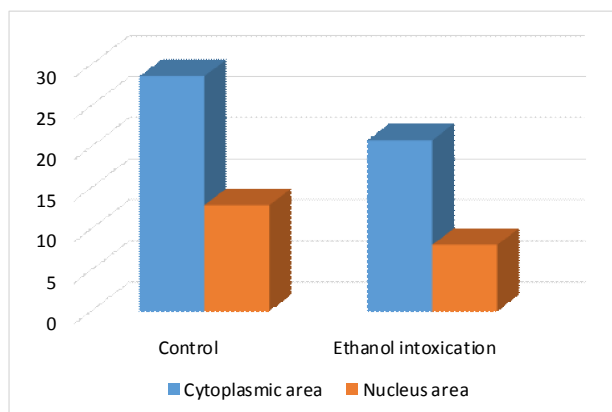


Fig. 4. Comparison of mean values of T2P morphometric parameters in control and experimental groups, μm^2 .

Table 2. Morphometric parameters of T2P in the control group of animals and under the influence of ethanol.

Group of animals	Cytoplasm area, μm^2 (M \pm m, n=30)	Nuclei area, μm^2 (M \pm m, n=30)	Nucleoli area, μm^2 (M \pm m, n=30)
Control	28,64 \pm 1,65	12,94 \pm 0,62	Not determined
Ethanol intoxication	20,85 \pm 1,94*	8,110 \pm 0,800*	Not determined

Notes: * reliably with control ($p < 0,05$).

values of the nuclei area were $8.110 \pm 0.800 \mu\text{m}^2$, which is less than the control group by 37.33% ($p < 0.05$) (Fig. 4).

Discussion

According to the literature, changes in the morphometric parameters of the cell are significant in the morphofunctional assessment of the cell state. Thus, the processes of transcription and transformation of ribosomal RNA (R-RNA) are associated with the nucleus and nucleoli, and the size of the nucleoli correlates with the intensity of cellular protein synthesis [11]. When comparing the morphometric parameters of pinealocytes of the group of animals that were in conditions of ethanol intoxication, with the control group of animals, a significant increase in all parameters of T1P was found. According to the literature and studies of other authors, such changes in the size of T1P indicate an increase in the processes of synthesis and, accordingly, the functional activity of the pineal gland [3]. At the same time, given the general decrease in the number of cells and their sparse location in the parenchyma, the increase in morphometric parameters, namely the area of the nucleus, nucleolus and cytoplasm of T1P compared to similar parameters of control animals indicate high functional activity of cells in compensatory response. Our karyometric measurements of T2P correlate with the results obtained by J. Martinez-Salvador and co-

authors in the study of the effect of ethanol on the morphology of the pineal gland and indicate a decrease in the average area of the nucleus and nucleolus of both the peripheral and central zones of the parenchyma of the organ [15].

Information on the effect of ethanol on changes in the morphometric parameters of pinealocytes in the literature we have studied is limited [15]. However, our studies correlate with data from other researchers who studied changes in the morphology of the pineal gland in response to the influence of pathological factors of endogenous and exogenous origin [4, 6, 9, 13].

This experimental article differs from those published in this field in that it is the first to describe and analyze data on the effect of ethanol on changes in the quantitative ratio and morphometric features of rat pinealocytes. The conducted mathematical and statistical analysis allowed to systematize the obtained experimental data and to present the comparative characteristics of the studied structures in the norm and under the influence of ethanol intoxication.

The obtained morphological data can be used later in research during the development of drugs aimed at correcting the negative effects of alcohol intoxication, treatment of alcohol poisoning and increase the body's adaptive capacity. The obtained results allow to expand and deepen the knowledge about the effect of ethanol on different parts of the brain. In the future it is planned to investigate the condition of the vascular bed of the pineal gland in ethanol intoxication.

Conclusions

1. As a result of histological examination, it was found that 30-day modeling of ethanol intoxication is accompanied by pronounced changes in the morphometric parameters of pinealocytes.
2. Remodeling of pinealocytes in the form of their edema and vacuolation of the cytoplasm was established, which had an impact on the morphometric parameters of T1P.
3. The ratio of T1P and T2P changes, namely: the number of active T1P increases by 8.17%.
4. Significantly increase the parameters of T1P and decrease T2P. It was determined that the average values of the cytoplasm area of T1P increase by 54.55% ($p < 0.05$), the area of the nucleus increases by 61.32% ($p < 0.05$), and the area of the nucleolus by 32.84% ($p < 0.05$) compared with the control group. The area of the cytoplasm of T2P decreased by 27.2% ($p < 0.05$), and the area of the nucleus by 37.33% ($p < 0.05$). The detected morphometric changes indicate high functional activity of cells, which has a compensatory nature in response to apoptosis of pinealocytes.

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МОРФОМЕТРИЧНІ ОСОБЛИВОСТІ ПІНЕАЛОЦИТІВ ЩУРІВ В УМОВАХ ХРОНІЧНОЇ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

Пшиченко В.В., Черно В.С.

Етанолу притаманні хронобіологічні ефекти, які пов'язані з пригніченням процесів синтезу і секреції мелатоніну та порушенням циклу "сон-неспанья". Відомо, що етанол спричиняє фрагментацію сну через часті пробудження, подовжує неспанья, зменшує тривалість повільної фази сну. Водночас зміни морфології епіфізу в умовах хронічного впливу етанолу лишаються малодослідженими. Особливий інтерес викликають зміни основних морфометричних параметрів пінеалоцитів, оскільки вони є маркером функціонального стану епіфізу. Метою дослідження було вивчити особливості морфологічних змін пінеалоцитів щурів в умовах фізіологічної норми і при хронічній інтоксикації етанолом. Для досягнення мети нами було використано 20 лабораторних самців-щурів: контрольна та дослідна групи. Контрольна група перебувала за стандартних умов віварію. Для дослідної групи моделювали алкогольну інтоксикацію шляхом введення 40% розчину етанолу з розрахунку 12 мг/кг маси тіла внутрішньошлунково 4 рази на добу. Вивчення морфометричних параметрів пінеалоцитів проводили на 30 добу від початку дослідження. За результатами морфометричних вимірювань встановлено достовірне підвищення параметрів світлих пінеалоцитів та зменшення показників темних клітин. Визначено, що середні значення площі цитоплазми світлих пінеалоцитів збільшуються на 54,55% ($p < 0,05$), площа ядра збільшується на 61,32% ($p < 0,05$), а площа ядерця на 32,84% ($p < 0,05$) у порівнянні з контрольною групою. Площа цитоплазми темних пінеалоцитів зменшується на 27,2% ($p < 0,05$), а площі ядра на 37,33% ($p < 0,05$). Відмічено і зміни у співвідношенні світлих та темних пінеалоцитів. Встановлено збільшення кількості активних світлих клітин на 8,17%. Виявлені морфометричні зміни вказують на високу функціональну активність клітин, що має компенсаторний характер у відповідь на апоптоз пінеалоцитів.

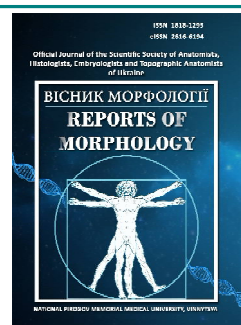
Ключові слова: епіфіз, хронічна інтоксикація етанолом, пінеалоцити, вакуольна дистрофія.



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Features of educational adaptation of young girls and young boys who are in institutions of higher medical education, using the distance format of the educational process

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The study of the peculiarities of educational adaptation of young girls and young boys of higher medical education, using the distance format of the educational process, is an important component of research that involves identifying risk factors for the likely development of negative changes in the adaptive resources of their body. The research, based on the application of questionnaires and structured interviews, was conducted on the basis of National Pirogov Memorial Medical University, Vinnytsya, where 110 students of 1-st and 3-rd courses were under supervision during the observation period. The analysis of the obtained data involved the application of descriptive statistics procedures based on the use of a package of statistical analysis applications "Statistica 6.1 for Windows" (licensed № BXXR901E245722FA). In the course of research the peculiarities of educational adaptation of young girls and young boys who are in the institution of higher medical education during the use of the distance format of the educational process are determined. During the analysis of the peculiarities of educational and, first of all, professionally-oriented education in a higher education institution, it was established that the level of academic success in all taught disciplines ($p < 0.05$) and in professionally-oriented subjects, in the distance learning format was higher than during the period of traditional classroom classes. It was found that distance learning helped to increase ($p < 0.05$) the level of nervous and emotional stress, and the main problems that arose during training in higher medical education in both options for organizing training and 1-st year and 3-rd year students mostly associated either with a feeling of constant fatigue, or with personal reasons. According to the physiological and hygienic assessment of the correlates of well-being of girls and boys at the end of school days in terms of distance learning format should be considered "bad", which is primarily due to the growing share of relevant data which is primarily due to the increase in the share of relevant data and a decrease in the share of indicators that marked the state of health as "good". The indicators regarding the peculiarities of the state of health and functional capabilities of the students' body should have been considered similar.

Keywords: young girls, young boys, educational adaptation, distance learning, hygienic assessment.

Introduction

The process of education of young girls and young boys, which takes place in a modern higher education institution, is a very special stage in the life of young people, clearly and directly related to the formation of a certain level of professional suitability and mastering the basics of clearly defined professional training and acquisition practical and significant professional experience. However, during this period, a young person, not yet fully ready to learn

meaningful techniques and professional skills, is left alone with the problems associated with the beginning of real adult life: a new environment, a new environment of peers and adults, quite often moving to another city, living in a dormitory, reviewing ideas about yourself that have formed so far, etc. [1, 7, 8, 9, 14, 15, 17, 22, 23].

This situation requires internal self-organization, stimulates the revision of existing and the identification

and awareness of new life values. Moreover, in the process of maintaining harmonious relations in the system "organism - social environment - environment", during which can change and the internal state of man and external parameters, the crucial role is played by such types of adaptation as mental (the process of establishing the optimal ratio of personality and environment in the course of performing activities that are inherent in man), psychophysiological (ensuring optimal organization of psychophysiological relationships and, consequently, maintaining both mental and physical health) and socio-psychological (an integral component of understanding and solving typical socially significant problems using situationally possible behaviors) adaptation [3, 7, 10, 13, 16, 18, 20].

Indeed, the process of adequate organization of mental, psychophysiological and socio-psychological adaptation is realized as a complex multilevel functional system, the regulation of which is associated with the activities of both psychological and physiological mechanisms, an important consequence of which is the formation of the necessary level of educational adaptation in fact, the process of human adaptation to being and active life in new, unusual, changing environmental conditions, accompanied by a number of negative phenomena: mental strain, insecurity, increased anxiety, and so on.. Especially if these processes are superimposed on a whole layer of no less unusual factors in the organization of the educational process, which saturated distance learning format, which is a set of modern information technologies that provide optimal pedagogical interaction between teachers and students at a distance interactively through communication, first of all modern Internet technologies, reproducing all components inherent in the educational process: goals, objectives, methods, organizational forms and forming a separate independent form of organization of the educational process, the main purpose of which is to ensure the mastery of both basic and additional educational programs. applicants directly at the place of residence or at the place of temporary residence [2, 5, 6, 7, 14, 15, 16, 21].

The aim of the study was to determine the features of educational adaptation of young girls and young boys who study in higher medical education, using a distance format of the educational process.

Materials and methods

Research aimed at conducting a comprehensive physiological and hygienic assessment of the level of educational adaptation of students of higher medical education in terms of distance learning, conducted on the basis of National Pirogov Memorial Medical University, Vinnytsya. 130 students were supervised during the observation period: 70 young girls and 60 young boys, who studied, respectively, in 1 (38 young girls and 30 young boys) and 3 (32 young girls and 30 young boys) courses.

Comprehensive assessment of the leading correlates of educational adaptation of student youth was carried out on the basis of studying the features of educational and, above all, professional-oriented, success and establishing the nature of education in higher education (first group of criteria), physiological and hygienic assessment of correlates of young girls and young boys during the study cycle (second group of criteria) and determination of the peculiarities of the state of health and functional capabilities of the body of students (the third group of criteria).

The analysis of the obtained data involved the application of descriptive statistics procedures based on the use of a licensed standardized package of statistical analysis applications "Statistica 6.1 for Windows" (licensed № BXXR901E245722FA).

Results

According to the first group of criteria that were determined, namely - data on the study of the characteristics of educational and, above all, professional-oriented, success and establishing the nature of education in higher education, it was necessary to note the following. During the analysis of the state and peculiarities of the previous adaptation of student youth to study in a higher medical institution, it was found that the largest share of respondents (about 1/2 of their total number) in the period before entering the university studied in a regular secondary school - respectively 52.6% of young girls and 46.6% of young boys who studied in the 1st year, and 53.1% of young girls and also 53.3% of young boys who studied in the 3rd year. Quite a significant number of students before studying at the Medical University were educated in specialized schools, lyceums and other educational institutions of medical and biological profile - their share was 44.7% among first-year young girls and 46.5% among young boys and 43.7% among third-year young girls and 40.0% among third-year young boys. Finally, in medical schools or colleges, only 2.6% of young girls and 6.6% of young boys in the first year, and 3.1% of young girls and also 3.3% of young boys who studied in higher medical education, entered the institution studied in the 3rd year.

In assessing the level of educational adaptation of students to the conditions of stay in a medical institution, it should be noted that the generalized level of academic success, in fact the level of success in all subjects taught, in the vast majority of subjects ranged from 4.0 to 4.5 points - such data were typical for 58.8% of first-year young girls and 43.3% of young boys, as well as 46.8% of third-year young girls and 36.6% of third-year young boys. The level of extremely high academic achievement, which ranged from 4.5 to 5.0 points and was typical for 18.4% of young girls and 10.0% of young boys enrolled in the 1st year, and for 28.1% of young girls and even 36.6% of young boys who studied in the 3rd year (Fig. 1). It was necessary to emphasize the fact that the level of academic success in the range from 3.5 to 4.0 points was also quite significant,

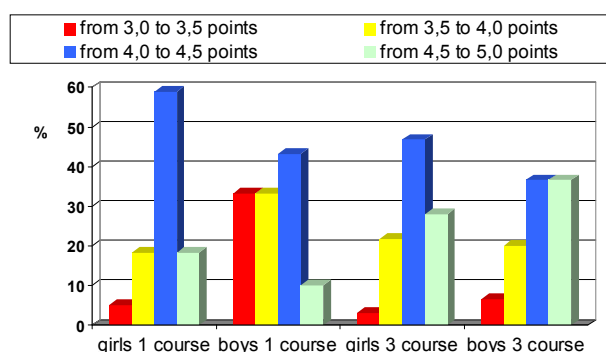


Fig. 1. Comparative diagrams of the level of success (scores) of young boys and young girls of 1st and 3rd courses according to the subjects taught in the institution of higher medical education.

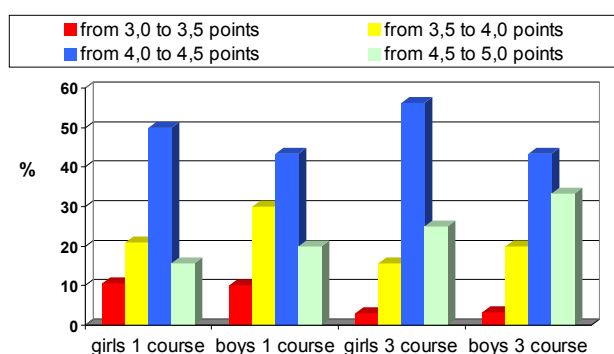


Fig. 2. Comparative diagrams of the level of success (scores) of young boys and young girls of the 1st and 3rd courses according to professionally oriented subjects, which were taught in the institution of higher medical education.

accounting for 18.4% of young girls first-year students and 33.3% of young boys first-year students, 21.8% of third-year young girls and 20.0% of third-year young boys. Finally, the academic achievement rate of up to 3.5 points was 5.2% among first-year young girls and 33.3% among first-year young boys and 3.1% among third-year young girls and 6.6% among third-year young boys.

At the same time, the level of academic success in professionally-oriented subjects, which primarily determines the level of acquisition of practical skills and abilities and the degree of professional adaptation of student youth, among the vast majority of respondents was also in the range from 4.0 to 4.5 points - these data were typical for 50.0% of first-year young girls and 43.3% of young boys and 56.2% of third-year young girls and 43.3% of young boys third-year students (Fig. 2). The level of extremely high academic achievement of 1st year students, which ranged from 4.5 to 5.0 points and was typical for 25.0% of young girls and 33.3% of young boys in 3rd year, should also be considered significant, as well as the level of academic success of 1st year students, which ranged from 3.5 to 4.0 points and was typical for 21.0% of young girls and 30.0% of young boys. At the same time, the level of academic success of 3rd year students, which ranged from 3.5 to 4.0 points, was 15.6% for young girls and 20.0% for young

boys, the level of academic success for 1st year students, which ranged from 4.5 to 5.0 points, was 18.4% for young girls and 16.6% for young boys. Finally, the level of academic success in vocational subjects in the range of up to 3.5 points was 10.5% among first-year young girls and 10.0% among young boys and 3.1% among third-year young girls and 3.3% among young boys third-year students.

The data that noted the level of practical skills defined by the educational program should be considered rather one-sided - among the representatives of all studied groups, despite the remote format of the educational process, its share gradually increased to 10.5% (less than 3.5 points), 15.7% (from 3.5 to 4.0 points) and 60.5% (from 4.0 to 4.5 points) among young girls of 1st year and 6.6% (less than 3.5 points), 30.0% (from 3.5 to 4.0 points) and 46.6% (from 4.0 to 4.5 points) among young boys of 1st year and making up 15.3% (less than 3.5 points), 18.7% (from 3.5 to 4.0 points) and 56.2% (from 4.0 to 4.5 points) among young girls of 1st year and 6.6% (less than 3.5 points), 26.6% (from 3.5 to 4.0 points) and 46.6% (from 4.0 to 4.5 points). At the same time, the level of practical skills acquisition should be considered too high only in 13.1% of young girls and 16.6% of young boys who studied in the 1st year, and 9.3% of young girls and 20.0% of young boys who studied in the 3rd year course.

15.7% of young girls and 3.3% of young boys who studied remotely in the first year, and almost twice as much, respectively 31.2% and 6.6% on the 3rd course noted as "too high" the level of nervous and emotional stress during their stay in a higher medical institution; as a large - 39.4% of young boys 1st year and 26.6% of first-year young girls and 40.6% of young boys third-year students and 40.0% of young girls third year students, as "moderate" - 34.2% of young girls and 50.0% of young boys who studied in the distance mode in the 1st year, and almost twice as many, respectively 21.8% of young girls and 40.0% of young boys of 3rd year study; as "a little tense" - 10.5% of first-year young boys and 16.6% of first-year young girls and 21.8% of third-year young boys and 13.3% of third-year young girls studied in the distance mode in the 3rd year. Only 1 young man (3.3%), a first-year student, defined the level of nervous and emotional stress during the educational activity as "absolutely insignificant".

At the same time, 2.6% of young girls and 20.0% of young boys studying in the first year, and 21.8% of young girls and 13.3% of young boys studying in the institution of higher medical education for 3 year noted as "too intense" the nature of education in higher education, as "tense" - 47.3% of young boys of 1st year and 43.3% of young girls of 1st year and 46.8% of young third-year students and 43.3% of third-year girls, as "moderately tense" - 42.1% of young girls and 23.3% of young boys who studied in the 1st year, and 18.7% of young girls and 33.30% of young boys who studied in the 3rd year, as "slightly tense" - 7.8% of young 1st year young boys and 10.0% of first-year young girls and 12.5% of third-year young boys and 10.0% of third-year

young girls (Fig. 3). As in the previous case, only 1 young man (3.3%), a first-year student, considered the regime of his/her own educational activity "completely unstressed".

The main problems that arose during the study in higher medical education and affected the level of educational adaptation, both first-year and third-year students were mainly associated with a feeling of constant fatigue and personal reasons (their own laziness, etc.) (Fig. 4). Such reasons were typical for 50.0% and 31.5% of young girls, respectively, and for 36.6% and 46.6% of young boys who studied in the 1st year, as well as, respectively, for 84.7% and 12.5% young girls and for 46.6% and 36.6% of young boys who studied in the 3rd year. The share of reasons of other content (poor health, level of teaching and pedagogical skills of teachers, family circumstances, etc.) was insignificant and did not exceed 15-17% among 1st year and 10-15% among third-year students.

Finally, during the hygienic assessment of the generalized level of adaptation of student youth to the distance learning format in higher medical education, it should be noted that the largest share of students assessed it as "satisfactory" and "good" - 34.2% and 42.2%, respectively young girls and 43.3% and 36.6% of young boys who studied in the 1st year, and respectively 28.1% and 40.6% of young girls and 23.3% and 53.3% of young boys who studied in the 3rd year. 15.7% of first-year young girls and 6.6% of first-year young boys and 15.6% of third-year young girls and 10.0% of third-year young boys considered the general level of adaptation to study to be "excellent", respectively 7.8% first-year young girls and 13.3% of young boys of 1st year study and 15.6% of third-year young girls and 10.0% of young boys third-year students as "unsatisfactory".

According to the second group of criteria that determined, namely the physiological and hygienic correlates of well-being of young girls and young boys during the study cycle, it was found that the well-being at the beginning of the majority of study days, the largest proportion of students - respectively 43.7% of young girls and 40.0% of young boys who studied in the 1st year, and 34.3% of young girls and 46.4% of young boys who studied in the 3rd year marked as "satisfactory", as "good" - respectively 28.9% of young girls and 26.7% of young boys who studied in the 1st year, and 25.0% of young girls and 36.7% of young boys who studied in the 3rd year. 5.2% of first-year young girls and 10.0% of first-year young boys and 6.2% of third-year young girls (no third-year young boy with such characteristics) reported their "excellent" health at the beginning of study days. Finally, at the beginning of the vast majority of study days, 13.1% of young girls and 16.7% of young boys who were in the first year, and 15.6% of young girls and 13.3% of young boys who studied in the 3rd year marked as "bad", as "very bad" - respectively 5.2% of young girls and 6.7% of young boys who studied in the 1st year, and 18.7% of young girls and 3.3% of young boys who studied in the 3rd year.

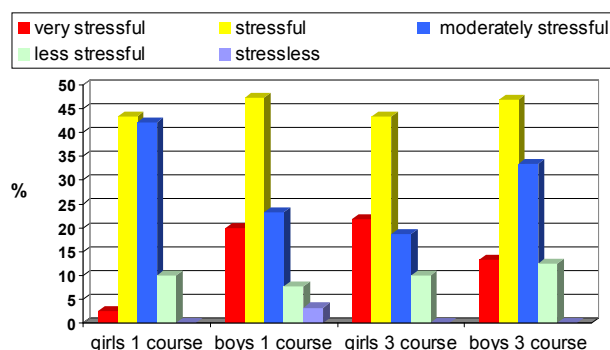


Fig. 3. Comparative diagram of indicators of the education nature (%) in young boys and young girls of 1st and 3rd courses in the institution of higher medical education.

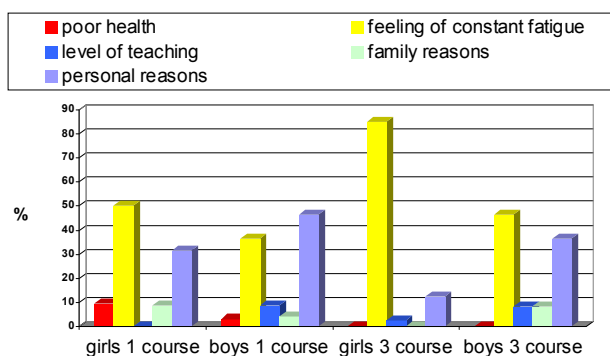


Fig. 4. Comparative diagrams of indicators of the main problems (%) that arise during training in a higher medical education institution in young boys and young girls of 1st and 3rd courses.

To a sufficiently pronounced extent, mainly directed in a favorable direction, the studied picture changed, which reflected the peculiarities of students' own feelings in the middle of study days. Thus, at this time the share of student youth who were in distance learning and noted their well-being as "satisfactory" was - respectively 55.2% of young girls and 50.0% of young boys who studied in the 1st year, and 53.1% of young girls and 40.0% of young boys who studied in the 3rd year; as "good" - respectively 31.6% of young girls and 33.3% of young boys who studied in the 1st year, and 31.2% of young girls and 40.0% of young boys who studied in the 3rd year. 5.2% of young girls of 1st year and 13.0% of first-year young boys and 3.3% of third-year young boys (no third-year young girls with such characteristics were registered) as "excellent" in the middle of study days. After all, at the beginning of the majority of study days, 5.2% of young girls who studied in the 1st year and 15.6% of young girls who studied in the 3rd year marked as "bad"; as "very bad" reported their feeling 2.2% of young girls and 3.3% of young boys who studied in the 1st year.

Indicators that registered and noted the peculiarities of students' own feelings at the end of study days were somewhat different from the previous ones - first of all they noticed a decrease in the share of indicators that marked well-being as "good" and an increase in the proportion of

data as "bad". As in all previous cases, at the end of the vast majority of study days, the largest share of students who were in distance learning, described as "satisfactory" - respectively, 47.3% of young girls and 50.0% of young boys who studied in the 1st year, and 50.0% of young girls and also 50.4% of young boys who studied in the 3rd year. At the same time, 23.6% of young girls and 23.3% of young boys who studied in the 1st year, and 15.6% of young girls and 15.6% of young boys who studied in the 3rd year, noted it as "good". At the beginning of the school days, 10.5% of young girls of 1st year and 16.6% of first-year young boys and 9.3% of third-year young girls and 9.3% of third-year young boys noted that they felt "excellent". Instead, at the end of the vast majority of study days, 15.7% of young girls and 6.7% of young boys in the first year, and even 21.8% (more than 1/5 of the total number of surveyed persons) young girls and also 21.8% (more than 1/5 of the total number of surveyed persons) of young boys who studied in the 3rd year reported feeling unwell as "bad"; as "very bad" - respectively 2.6% of young girls and 3.3% of young boys who studied in 1 course, and 3.1% of young girls and also 3.1% of young boys who studied in the 3rd year.

At the same time, it should be noted that at the beginning of the study week, the largest share of students who were in distance learning, also noted as "satisfactory" - respectively 39.4% of young girls and 36.7% of young boys who studied at 1st year, and 34.3% of young girls and 40.0% of young boys who studied in the 3rd year, and as "good" - respectively 21.0% of young girls and 30.0% of young boys who studied in the 1st year, and 31.2% of young girls and 43.3% of young boys who studied in the 3rd year. At the beginning of the study week, a much larger number of first-year students and a much smaller number of third-year students, namely 21.0% of young girls of 1st year and 16.6% of first-year young boys, and only 6.2% of third-year young girls and only 3.3% of third-year young boys marked it as "excellent". Finally, at the beginning of the school week, 13.1% of young girls and 10.0% of young boys who studied in the first year, and 9.3% of young girls and 10.0% of young boys who studied in the third year, noted their own state of health as "bad", as "very bad" - respectively 5.2% of young girls and 6.7% of young boys who studied in the 1st year, and 18.7% of young girls and 3.3% of young boys who studied in the 3rd year.

Almost similar to the previous ones were the results determined among the representatives of the studied groups in the middle of the study week. Thus, at this time the proportion of student youth who were in distance learning and noted their well-being as "satisfactory" was - respectively 36.8% of young girls and 53.0% of young boys who studied in the 1st year, and 31.2% of young girls and 50.0% of young boys who studied in the 3rd year, as "good" - respectively 39.4% of young girls and 33.3% of young boys who studied in the 1st year, and 40.6% of young girls and 43.3% of young boys who studied in the 3rd year. In the middle of the study week, 7.8% of young girls 1st year and

33% of first-year young boys and 3.1% of third-year young girls and 3.3% of third-year young boys (no third-year young girls with such characteristics registered). Finally, in the middle of the study week, 10.5% of young girls who studied in the 1st year and 3.3% of young boys who studied in the 1st year, and 21.8% of young girls who studied in the 3rd year, and 3.3% of boys who studied in the 3rd year marked it as "bad"; as "very bad" - respectively 5.2% of young girls and 6.7% of young boys who studied in the 1st year, and 1.2% of young girls who studied in 3rd year (no young third-year young boy student with such characteristics of health was registered).

At the end of the study week, the largest share of student youth who were in distance learning, noted as "satisfactory" - respectively 44.7% of young girls and 23.3% of young boys who studied in the 1st year, and 25.0% of young girls and also 36.6% of young boys who studied in the 3rd year; as "good" - respectively 21.0% of young girls and 33.3% of young boys who studied in the 1st year, and 18.7% of young girls also 30.9% of young boys who studied in the 3rd year, and, unlike most previous cases, as "excellent" - respectively 31.5% of young girls and 33.3% of young boys who studied in the 1st year, and 40.6% of young girls also 33.3% of young boys who studied in the 3rd year. At the same time, at the end of the study week, 6.7% of young boys who studied in the 1st year and 15.6% of young girls who studied in the 3rd year reported their feeling as "bad", and as "very bad" respectively 2.6% of young girls and 3.3% of young boys who studied in the 1st year.

Finally, at the end of the weekend, the largest proportion of 1st year students who were in distance learning, noted as "good" and "excellent" - respectively 29.9% and 28.9% of young girls and 46.7% and 23.3% of young boys, at the same time, the largest share of third-year students who were in distance learning, noted as "satisfactory", "good" and "excellent" - respectively 21.8%, 28.1% and 21.8% for young girls and 33.3%, 33.3% and 20.0% for young boys. At the end of the weekend, 21.0% of first-year young girls and 6.6% of young boys of 1st year and 18.7% of third-year young girls and 10.0% of third-year young boys reported their "bad" health, and as "very bad" - respectively 2.6% of first-year young girls and 6.6% of young boys of 1st year and 9.3% of third-year young girls and 3.3% of young boys third-year students.

According to the last third group of criteria, namely: the peculiarities of the state of health and functional capabilities of the body of students, the following results were obtained. Thus, during an in-depth hygienic assessment of the prevalence of diseases with temporary disability, it was found that 4 or more times a year, as a rule, sick - 3.3% of first-year young boys and 12.5% of third-year young girls, 3 times a year, usually sick - 18.4% of young girls and 10.0% of young boys who studied in the 1st year, and 25.0% of young girls and 13.0% of young boys who studied in the 3rd year, 2 times a year, as a rule, 18.4% of first-year young girls and 43.3% of first-year young boys and 31.2% of third-year young

girls and 10.0% of third-year young boys; and were ill, as a rule 1 time, 44.7% young girls and 30.0% of young boys who studied in the 1st year, and 18.7% of young girls and 40.0% of young boys who studied in the 3rd year, in the end, as a rule, did not get sick at all - 26.3% of first-year young girls and 13.3% of first-year young boys and 12.5% of third-year young girls and 36.7% of third-year young boys.

The predominant duration of the disease with temporary disability, which was registered, should be considered the duration of the disease in the range from 3 to 7 days, which was typical for 55.2% of young girls and 36.7% of young boys who studied in the 1st year, and for 62.5% of young girls and 56.7% of young boys who studied in the 3rd year. In addition, the share of indicators that reflected the duration of acute illness within 3 days and within 7 to 14 days - 26.6% and 15.7% among first-year young girls, 10.0% and 30.0% among first-year young boys and 9.3% and 18.7% among third-year young girls, 23.3% and 13.3% among third-year young boys.

The presence of chronic diseases kept pronounced impact on the adaptive capacity of the body, worsening the processes of forming a high level of both physical and mental performance, according to the official statistics indicate (based on medical examinations) 15.8% of young girls and 23.3% of young boys who studied in the 1st year, and 28.1% of young girls and 23.3% of young boys who studied in the 3rd year. According to a subjectively significant structured survey, their number increased by 2-2.5 times, amounting to 31.3% in first-year young girls and 40.0% in young boys 1st year and 43.4% in third-year young girls and 45.0% for young boys third-year students.

Analyzing the peculiarities of the frequency of exacerbations of diseases with chronic pathological process, it should be noted that more than 4 times a year exacerbation of chronic diseases was observed in only 5.6% of first-year young girls and 3.1% of third-year young girls, 3 times a year - among 7.6% of first-year young girls and among 3.3% of young boys first-year students, 2 times a year - 7.8% of first-year young girls and 13.3% of young boys first-year students and 12.5% of third-year young girls, 1 once a year - among 23.6% of first-year young girls and 13.3% among young boys and among 12.5% of third-year young girls and among 13.3% of young boys third-year students. Exacerbations of diseases with a chronic course of the pathological process were not registered in 60.5% of first-year young girls and 70.0% of first-year young boys and 71.8% of third-year young girls and 86.6% of third-year young boys who studied with the use of distance learning forms of education.

Finally, when assessing the peculiarities of the subjectively significant attitude of student youth to their own health, it was found that 2.6% of young girls who studied in the 1st year considered it "very bad", 3.1% of young girls considered it "bad" who studied in the 3rd year, "satisfactory" - 34.2% of young girls and 20.0% of young boys who studied in the 1st year, and 40.6% of young girls and 13.3% of

young boys who studied in the 3rd year, "good" - the largest share of respondents, namely: 52.6% of young girls and 56.6% of young boys, as students in the 1st year, and 50.0% of young girls and 70.3% of young boys, as students in the 3rd year, "excellent" - 10.5% of young girls and 23.3% of young boys studied in the first year, and 6.2% of young girls and 16.6% of young boys studied in the third year.

Discussion

According to a number of studies conducted in recent years [3, 7, 11, 14, 17, 19, 21], the process of adequate determination of the level of educational adaptation of students is impossible without an in-depth hygienic assessment of health, and thus establishment of both subjectively (according to the data of structured surveys and questionnaires) and objectively significant (according to the data on morbidity indicators, parameters of educational-significant psychophysiological, mental and socio-psychological adaptation of young girls and young boys) of its indicators.

In the course of research, during the consideration of the first group of criteria (features of educational and, above all, professional-oriented, success and nature of education in higher education), it was found that the level of academic success in all disciplines taught and for professional oriented subjects, in the distance learning format was higher than during the period of traditional classes. And, first of all, it concerned the generalized results of educational activity ($p < 0.05$). In particular, if in our study the level of academic success, which ranged from 4.5 to 5.0 points, was typical for 18.4% of young girls and 10.0% of young boys who studied in the 1st year, and for 28.1% of young girls and even 36.6% of young boys who studied in the 3rd year, according to the data obtained in the study of V.M. Moroz and co-authors [9], similar indicators were characteristic only for 7.8% of young girls and 6.0% of young boys of 1st year students and 16.6% of young girls and 12.0% of young boys of 3rd year students.

Instead, data that reflected the level of practical skills defined by the educational program should be considered completely unidirectional in the course of online learning. Thus, among the representatives of all studied groups, despite the remote format of the organization of the educational process, the indicators of the level of acquisition of practical skills gradually increased. Interestingly, the data obtained among young girls and young boys who were in off-line training should be considered almost similar. As a very high level of neuro-emotional stress during the training in higher medical education in remote mode indicated 15.7% of young girls and 3.3% of young boys who studied at 1 year, and almost twice as much as 31.2% of young girls under and 6.6% of young boys who studied in the 3rd year. Such results exceeded the data obtained in studies of similar content among students who studied in the classroom [9, 17]. Thus, distance learning contributed to an increase ($p < 0.05$) in the level of nervous and emotional

stress during the acquisition of knowledge in a higher medical education institution.

Slightly more intense, but statistically insignificant ($p > 0.05$), should be considered the nature of educational activities. Thus, under the conditions of distance learning, 22.6% of young girls and 20.0% of young boys who studied in the 1st year, and 21.8% of young girls and 13.3% of young boys who studied in the 3rd year noted it as too intense. At the same time, according to the data [9, 16], 32.1% of young girls of 1st year and 8.0% of first-year young boys, 15.7% of third-year young girls and 6.0% of young third-year students noted as an extremely intense nature of education in the conditions of classroom training in a higher medical education institution.

The main problems that arose during their studies at the institution of higher medical education and affected the level of educational adaptation, in both variants of the organization of education, both first-year and third-year students associated either with a feeling of constant fatigue or with personal reasons (loss motivation, own laziness, careless attitude to the performance of educational duties, etc.).

According to the data inherent in the second group of criteria (data of physiological and hygienic assessment of the correlates of well-being of young girls and young boys during the study cycle), at the end of study days in the distance learning format, first of all, a decrease in the proportion of indicators that indicated well-being as well against the background of an increase in the share of data that testified to their own well-being as "bad". However, the obtained data did not reach the level inherent in the indicators characteristic of the classroom format of educational activities. Thus, in the first case, actually feel the at the end of the overwhelming number of study days as "bad" marked - 15.7% of young girls and 6.7% of young boys ($p < 0.05$), who studied for 1 year, and even 21.8% of young girls and also 21.8% of young boys who studied in the 3rd year. At the same time in the second case, according to data [9, 17], as "bad" their health was characterized by 19.6% of young girls and 28.0% young boys in the first year and 29.4% of young girls and 20.0% of young boys in the third year. At the same time, at the end of the weekend, the share of students with the same health characteristics equalized ($p > 0.05$).

The indicators determined by the third group of criteria

(features of the state of health and functional capabilities of the students' body) should be considered quite similar ($p > 0.05$).

In the future, the results should be used both to develop methods of hygienic assessment of adaptive resources of students, taking into account the peculiarities of their educational activities, and during the scientific substantiation of effective health technologies in higher education.

Conclusions

1. In the course of the conducted researches the peculiarities of educational adaptation of young girls and young boys who are in the institution of higher medical education, when using the remote format of the organization of educational process are defined.

2. In the course of the analysis of features of educational and, first of all, professionally-oriented success and character of training in institution of higher education it is established that the level of educational success both on all disciplines taught ($p < 0.05$), and on professionally-oriented subjects, in the distance learning format was higher than during the period of traditional classes. Instead, data that noted the level of practical skills defined by the educational program should be considered completely unidirectional during online learning - among the representatives of comparison groups, despite the remote format of the educational process, its share gradually increased.

3. It was found that distance learning helped to increase ($p < 0.05$) the level of nervous and emotional stress, and the main problems that arose during training in higher medical education and affected the level of educational adaptation in both options for learning for 1st year and third-year students mostly associated either with a feeling of constant fatigue, or with personal reasons.

4. According to the physiological and hygienic assessment of the correlates of well-being of young girls and young boys at the end of study days in terms of distance learning, first of all, the decrease in the share of indicators that marked health as "good" and the growing proportion of evidence own well-being as "bad". Similar ($p > 0.05$) should be considered indicators that were determined according to a group of criteria for the characteristics of the state of health and functionality of students.

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ОСОБЛИВОСТІ НАВЧАЛЬНОЇ АДАПТАЦІЇ ДІВЧАТ І ЮНАКІВ, ЯКІ ПЕРЕБУВАЮТЬ В ЗАКЛАДАХ ВИЩОЇ МЕДИЧНОЇ ОСВІТИ, ПІД ЧАС ВИКОРИСТАННЯ ДИСТАНЦІЙНОГО ФОРМАТУ ОРГАНІЗАЦІЇ НАВЧАЛЬНОГО ПРОЦЕСУ

Вергелес Т.М.

Вивчення особливостей навчальної адаптації дівчат і юнаків, які перебувають у закладах вищої медичної освіти, під час використання дистанційного формату організації навчального процесу, є важливим компонентом наукових досліджень, котрі передбачають встановлення чинників ризику щодо ймовірного розвитку негативних зрушень адаптаційних ресурсів їх організму. На підставі застосування методик анкетування та структурованого інтерв'ювання дослідження проводили

на базі Вінницького національного медичного університету імені М.І. Пирогова, де під наглядом всього періоду спостережень знаходились 110 студентів 1 і 3 курсів. Аналіз отриманих даних передбачав застосування процедур описової статистики на основі використання пакету прикладних програм статистичного аналізу "Statistica 6.1 for Windows" (ліцензійний № ВХХR901E245722FA). Під час дослідження визначені особливості навчальної адаптації дівчат і юнаків, котрі здобувають знання в закладі вищої медичної освіти, під час використання дистанційного формату організації навчального процесу. Аналіз особливостей навчальної і, передусім професійно-орієнтованої, успішності та характеру навчання у закладі вищої медичної освіти встановив, що рівень навчальної успішності як за всіма дисциплінами, що викладались ($p < 0,05$), так і за професійно-орієнтованими навчальними предметами, у дистанційному форматі навчання був вищим, ніж впродовж періоду проведення традиційних аудиторних занять. Виявлено, що навчання у дистанційному форматі сприяло підвищенню ($p < 0,05$) рівня нервово-емоційного напруження, причому основні проблеми, які виникали в ході навчання у закладі вищої медичної освіти за обох варіантів організації навчання і студенти-першокурсники, і студенти-третьокурсники переважно пов'язували або з почуттям постійної втоми, або з особистісними причинами. Згідно з даними фізіолого-гігієнічної оцінки корелят самопочуття дівчат і юнаків наприкінці навчальних днів за умов дистанційного формату навчальної діяльності слід вважати як "погане", що, насамперед, обумовлене зростанням питомої ваги відповідних даних та зменшенням частки показників, котрі відзначали самопочуття як "добре". Подібними слід було вважати і показники щодо особливостей стану здоров'я та функціональних можливостей організму студентів.

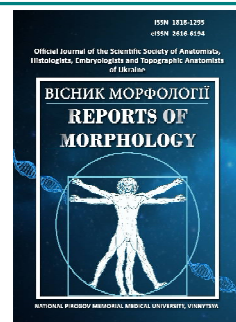
Ключові слова: дівчата, юнаки, навчальна адаптація, дистанційне навчання, гігієнічна оцінка.



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Macro-microscopic changes in the kidneys of the rats affected by methyl tertiary butyl ether in different time of the research

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Methyl tert-butyl ether (MTBE) is a relatively new anthropogenic factor of environmental pollution. It is already known that it is an environmentally hazardous substance that has a toxic effect on the human body. The purpose of the research is to study the effect of MTBE on the structural organization of the kidneys of research animals. White outbred rats were involved into the research, which were treated with 500 mg/kg MTBE in an oil solution, which was administered intragastrically using a gastric catheter. Structural changes in the kidneys on the 3rd, 8th, 15th, 22nd and 60th day of the research were studied by means of light-optical and electron microscopy. The obtained material was processed statistically using the parametric Student's test and the nonparametric Kolmogorov-Smirnov test in accordance with the principles of variation statistics. During the runtime of the research, we have clearly defined the staging of changes in the structural organization of the kidney and the dependence of their severity on the duration of MTBE action. With short-term exposure (3, 8 and 15 day of the research), the weight of the kidneys and other organometric parameters do not differ from those in the control group. In the initial stages of MTBE action, we have observed reactive changes, which are clearly manifested in the structures of the filtration barrier (3rd day), and with an increase in the observation period (8th day) they spread to the epithelial cells of the proximal tubules. After 15 days of MTBE action, against the background of pronounced compensatory-adaptive events, we have noticed the development of dystrophic-destructive processes in the renal corpuscles and in the proximal tubules. There are structural signs of enhanced synthetic activity in the mesangial cells. On the 22nd day of the experiment, the morphometric index of the mass and linear dimensions of the kidneys increase in comparison with the control group. There are structural signs of atrophy and hypertrophy processes in the nephrons. There are progressive changes in the tubular apparatus of the kidneys in comparison with the previous period of the research. Long-term action of MTBE (60 days) causes further deepening of dystrophic and destructive changes in all parts of the nephron, which differ in intensity. It has been proved that MTBE has a negative effect on all structural components of the nephron, which leads to disorganization of the filtration and reabsorption of kidney apparatus and significant development of the connective tissue, which causes the development of sclerosis. Besides, it has been proved that MTBE has an apparent nephrotoxic effect and the degree of damage to the structural components of the kidney is determined by the duration of the body's contact with this toxic substance.

Key words: kidneys, Methyl tert-butyl ether, nephron.

Introduction

Environmental monitoring determines the deterioration of the environment and, primarily, due to environmental pollution by various chemical compounds [23, 26, 27]. Various factors of physical, chemical and biological genesis that constantly surround a person, directly negatively affect the organs and systems of the body, resulting in the

development of various pathological conditions [3, 11, 12]. One of the global pollutants, especially in modern cities, where transport infrastructure is significantly developed, is the exhaust gases of cars [4, 9]. Exhaust gases contain more than 300 different chemical compounds, most of which are toxic to the environment. One of the components of exhaust

gases is Methyl tert-butyl ether (MTBE), which is used to increase the octane number of gasoline [1, 19, 24]. Many studies have been conducted on the effects of various toxic components of exhaust gases on humans and laboratory animals in the experiment [13, 14, 15]. MTBE is a relatively new anthropogenic factor, but it is already known that it is an environmentally hazardous substance that pollutes the environment [8, 17, 28]. The impact of MTBE on various organs and systems of humans and experimental animals has been studied in a few studies [7, 18]. It is established that MTBE has a negative effect on the body and causes the development of allergic, respiratory, cancer, lesions of the nervous system and a number of other pathological conditions [20, 28]. The selection of the bulk of chemical agents that enter the human body from the environment is carried out by the kidneys and therefore the kidneys are primarily a target for toxic chemicals [19, 21]. In addition, the kidneys are affected by the breakdown products of chemical components, which usually have a nephrotoxic effect. The effect of MTBE on the structure of the kidney in the experiment is practically not studied. Of particular importance is the study of the toxic effects of MTBE in the late stages of the experiment, because, as a rule, the human body is exposed to long-term exposure to toxic substances [2, 29].

The aim of the work is to study at the macroscopic, light-optical and ultrastructural levels the morphological changes of the kidneys under the influence of MTBE at different terms of the experiment.

Materials and methods

The studies were performed on white outbred rats of adult age, which were kept in standard conditions vivarium of the National Medical University named after O.O.Bogomolets. Animals received 500 mg/kg MTBE daily in oil solution, which was administered intragastrically by gavage. The control group consisted of white outbred rats, which were also kept (similar to the experimental group) in standard vivarium conditions and were not exposed to MTBE.

The provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1985) and the "General Ethical Principles for Animal Experiments", approved by the First National Congress on Bioethics, were followed in keeping, caring for and manipulating all animals. (Kyiv, 2001).

Animals were removed from the experiment under ether anesthesia 3, 8, 15, 22 and 60 days after its onset by decapitation according to the "Guidelines for the Removal of Animals from the Experiment" (1985) and the kidneys were immediately removed. In order to detect pathological changes of the kidneys at the macroscopic level, their appearance was determined, the mass and linear dimensions of the organ were measured. The material was fixed in 10% neutral formalin, paraffin sections were stained with hematoxylin and eosin.

For electron microscopic examination, 1 mm³ kidney pieces were removed. Processing of material for electron

microscopic examination was carried out according to conventional methods. On the LKB III ultratome (Sweden), semi-thin and ultra-thin sections were obtained from the blocks. Semi-thin sections were stained with toluidine blue. Ultrathin sections were contrasted with 2% uranyl acetate solution and lead citrate. Samples were examined under an electron microscope PEM-125K at magnifications of 6-20 thousand, followed by photography.

Results

After 3 days of the experiment, the macroscopic structure of the kidneys, their weight and organometric parameters did not differ from those of the control group. According to the optical examination, it was found that on day 3 of the action of MTBE, cortex and medulla of the kidney were well differentiated. Vascular glomeruli were mostly the same size, unchanged, their capsule is not thickened. Urinary spaces are free. However, in the kidneys there were some local changes, manifested by focal edema, dilation of the lumen of blood vessels, especially the venular, with accumulation of blood cells, plethora of individual glomeruli and focal edema of the tubular epithelium. At the ultrastructural level, changes were detected in all components of the nephron. The cytoplasm of endothelial capillaries of glomeruli of high electron density, swollen. In endothelial cells, the zonation of the cytoplasm is preserved: the zone of the perikaryon and the delicate peripheral areas are determined, in which separate thickened cytoplasmic islands protruding into the lumen of the vessel are observed. Organelles of the synthetic apparatus are located in the area of the perikaryon and thickened cytoplasmic islets. The microvesicular transport system is poorly developed. Micropinocytic vesicles are filled with electronically transparent contents. Thinned peripheral areas of endotheliocytes contain open fenestra, the size of which varies. The basement membrane is predominantly of moderate electron density, without significant changes (Fig. 1). Cytopodia

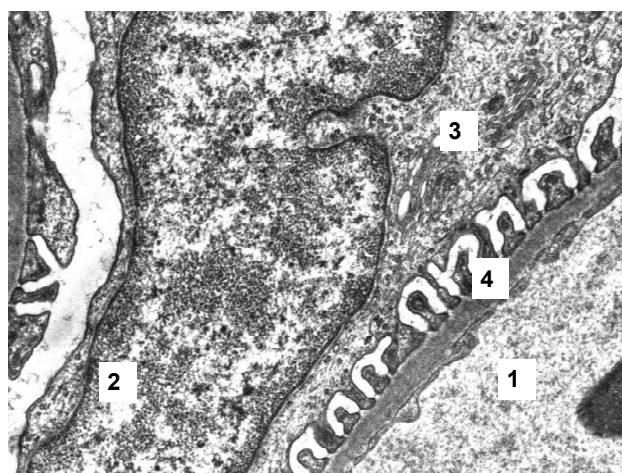


Fig. 1. Ultrastructure of the renal corpuscle of rats after 3 days of MTBE use. Gloss of the glomerular capillary (1). Nucleus (2), Golgi complex (3) in podocytes. Glomerular basement membrane (4). x18000.

of podocytes are variable in shape and size: short-shortened cytopodia are determined by length, other elongated, located at different angles to the basement membrane. In many cytopodia, small electron-dense inclusions are identified at the basal end. Diaphragms are visualized in almost all filtration slits.

The ultrastructure of mesangial cells indicates the activation of biosynthetic processes, signs of which are hypertrophy of their organelles, primarily the tubules of the endoplasmic reticulum.

The brush border was disturbed in the proximal tortuous tubules of the nephron. The microvilli that form it locally lost their parallel orientation, fragmented, and desquamated into the lumen of the tubule, as a result of which the areas of the apical surface of epitheliocytes sometimes did not have such a border. Epitheliocytes are mosaic by ultrastructural structure. Electronically illuminated cells with varying degrees of edema are observed. The consequence of significant violations of the excretion of water from the cells is the formation of sequestration with edematous fluid, obstructing the lumen of these tubules. In other epitheliocytes there is an accumulation of fine material and, depending on its compaction, the cells vary in electron density. A small number of apoptotic epitheliocytes are identified, which have already lost contact with other cells and have been pushed into the lumen of the tubule.

On the 8 day of the experiment, the macroscopic structure of the kidneys, their weight and organometric parameters did not change and did not differ from those of the control group. At the optical level, the glomeruli are mostly the same size, in some there was an expansion of the urinary space at one of the poles. The glomerular capsule is not thickened. Glomerular capillaries are full-blooded. In some hypertrophied glomeruli there is hypercellularity and expansion of mesangial spaces. More significant changes were found in the tubular apparatus of the kidneys. In the cortex, in the proximal and distal tubules of the kidneys there is granular and hydropic dystrophy of epitheliocytes, their desquamation into the lumen of the tubules and the destruction of the basement membrane in some places. At the ultrastructural level, cells with slightly increased electron density of nuclei and cytoplasm predominate among glomerular capillary endothelial cells. Peripheral areas, as a rule, are thinned and separate microclasmatous outgrowths are noted. Non-diaphragm fenestrae are uneven, sometimes their length is quite significant, which gives reason to think about the difference in endothelial cells. Podocytes undergo further changes. The number of organelles in the body and cytotrabeculae decreases slightly compared to the previous observation period. Cytopodia of podocytes are characterized by the accumulation of electron-dense material, which occupies a significant area. The largest changes among the structures of the renal corpuscle undergo mesangial cells, the number of which is increasing. Two mesangial cells are sometimes observed in the bifurcations of capillary loops. Some mesangiocytes have

ultrastructural signs of increased biosynthetic activity. They contain a nucleus with chromatin and nucleolus evenly distributed throughout the karyoplasm, a significant number of ribosomes, endoplasmic reticulum tubules, large mitochondria with a transparent matrix and cristae, microfibrils. Changes in proximal tubules of a nephron amplify. Dark cells are common among epitheliocytes. The microvilli on the apical surface are mostly fragmented, partially desquamated, and the number of secretory vesicles and associated granules is reduced. At the same time, light cells are observed, in the cytoplasm of which are placed single mitochondria, tubules of the endoplasmic reticulum, ribosomes, secretory granules, which are sometimes grouped, forming structures resembling residual bodies in dark cells. The distal tubules, in which epitheliocytes of moderate electron density are widespread, suffer to a lesser extent.

On the 15 day of the experiment, the macroscopic structure of the kidneys, their mass and organometric parameters did not differ statistically from those of previous terms and the control group. At the optical level, structural changes in some renal corpuscles, represented by the phenomena of hypertrophy, as well as atrophy and edema of the glomeruli, were determined. At the electron microscopic level, the structural components of the nephron became more mosaic. Part of the body retains the integrity of the structural components and signs of functional activity. But even in such bodies, the lumens of the capillaries are partially filled with erythrocytes and coagulated plasma, which can lead to a slowing of the flow rate. In cytotrabeculae the number of organelles is insignificant, sometimes they have electronically dense deposits. Similar deposits are found in individual cytopodia of different sizes. Along with renal corpuscles with ultrastructural signs of functional activity, corpuscles are observed in the cells of which destructive-dystrophic processes predominate. The lumen of the glomerular capillaries of such bodies is unevenly expanded, deformed, sometimes shaped blood elements and felt-like accumulations are determined.

In the electron-dense cytoplasm of endothelial cells, a small number of organelles, especially mitochondria, are detected. Thinned peripheral areas of the endothelial cell cytoplasm are fragmented in places and desquamated into the lumen of the capillary. The areas between the fragments of the cytoplasm of endothelial cells are filled with fine-grained electron-dense contents, sometimes there is the formation of growths of the basal surface of endotheliocytes, which are immersed in the periendothelial space or surrounded by the basement membrane. Edema of the periendothelial space is determined, which is filled with fine-grained substance of medium electron density, as a result of which the basal surface of endothelial cells is located at a certain distance from the basement membrane, which in such places is thickened, expanded, heterogeneous in structure, compacted. In other areas, the basement membrane is homogeneous and formed by a solid material

of high electron density. Significant structural changes are defined in podocytes. Cytotrabeulae are different in size, uneven in electron density; organelles are practically absent in the cytoplasm of cytotrabeula. In some podocytes the phenomena of necrosis are determined and they are desquamated into the lumen of the nephron capsule. The number of cytopodia decreases, they are irregularly located along the basement membrane, variable in size and shape; shortened, thickened cytopodia with an enlightened cytoplasmic matrix are determined. Other cytopodia are long, thin, electronically dense, located at different angles to the basement membrane, sometimes cytopodia are closely adjacent to the basement membrane. Filtration slits are located irregularly, of various sizes; some of them are covered with thickened filter diaphragms. Filtration slits are determined, where the destruction of their filtration membranes is determined. Mesangial cells contain a functionally active nucleus with uniformly distributed chromatin, around which most mitochondria are located. The processes of mesangial cells are filled with ribosomes, polysomes, tubules of the endoplasmic reticulum, microfibrils. In the proximal tubules, a significant mosaic of the ultrastructure of the epitheliocytes lining them is determined. There are tubules, the lumens of which are filled with microvilli that are close to each other, until they merge. In the apical part of epitheliocytes there are typical vesicles, secretory granules, primary lysosomes. Below, to the basal surface of the nucleus with a fairly evenly distributed chromatin, numerous mitochondria, lipid-loaded and large lysosomes with lamellar or uneven electron seals. Mitochondria in these cells have clearly structured outer membranes and cristae. All this indicates their active functional activity. However, there are tubules, where the microvilli of the bristle border are not dense, are at different stages of fragmentation and desquamation. The lumens of the proximal tubules are densely filled with sequesters with denatured proteins and edema fluid. As a rule, in cells with the changed microvilli the reduced quantity of organelles is defined, the cytoplasm is electronically condensed. The pycnomorphic nucleus is located in some dark cells, and the epitheliocytes themselves lose lateral contacts and separate from each other. There are also tubules, where the cells changed by the dark type are reduced in size and in the form of small electronically dense clusters are located in the lumen of the tubules. Such changes indicate an apoptotic pathway of epithelial cell death. In this case, the processes of apoptosis in the epitheliocytes of the proximal convoluted tubules occur asynchronously, a sign of which is the presence of cells at different stages of apoptosis and the appearance of apoptotic cells. A feature of the ultrastructure of epithelial cells of the proximal tubules in this period of the experiment is the presence of irregularly shaped structures, the electron density of the matrix which varies from transparent to moderately compacted, regardless of the degree of changes in the cells. These structures are residual bodies - the product of lysosomal dysfunction, which

accumulate and are not disposed of.

On the 22 day of the experiment there is an increase in the organometric parameters of the kidneys. Thus, the mass of the left kidney increases by 24%, and the right by 22% compared with the control group. Changes in the linear size of the kidney have a similar direction: the length, width, thickness of the organ are significantly increased in comparison with similar parameters of the control group. The histological structure of the glomeruli is not significantly changed in comparison with the previous terms of observation. Both atrophied and hypertrophied glomeruli are observed. Glomerular capillaries are dilated, full-blooded. In some glomeruli, the urinary space is dilated and filled with cellular detritus, which consists of endothelial and mesangial cells. Moderate sclerosis is observed around the capsule of individual glomeruli. The tubular apparatus of the kidneys undergoes more pronounced changes compared to the previous observation period. Individual tubules retain their structure, they contain cells in which the nuclei are almost not visualized, the cytoplasm of cells - with signs of granular and hydropic dystrophy. In many tubules, the lumen is completely filled with conglomerates of desquamated cells and cellular detritus. The boundaries of the vast majority of tubules are not defined. There is a pronounced swelling of the interstitium, there are plasma cells and fibroblasts. According to ultrastructural analysis, the destructive-dystrophic changes that develop in the renal corpuscles have a different direction than in previous terms of the experiment. There is no dark, apoptotically altered endothelial cells and podocytes. Peripheral areas of endothelial cells are mostly thinned, sometimes to the thickness of two membranes. Fenestra in thin areas are uneven and vary in length. In some places, there are wide loci between adjacent fragments of the cytoplasm, as well as diaphragmatic fenestrae, which appear in pathological conditions. The prevalence of microclasmatous growths on the luminal surface of endothelial cells and in the lumen of capillaries, as well as large sequesters with edematous fluid (Fig. 2) is noteworthy.

Podocytes change according to the light type: nuclei predominate, where chromatin forms clusters of insignificant electron density, and karyoplasm is electronically transparent. In the same enlightened cytoplasm are organelles, the number of which is reduced compared to the previous term of the experiment. Cytopodia are diverse in shape and ultrastructure. Some of them retain their characteristic leg-like shape and typical diaphragmatic fissures, and other cytopodia acquire an elongated shape or protrude into the urinary space, spreading along the glomerular membrane. A significant number of mesangial cells are identified, which are located at the bifurcations of capillary loops and contain 1-2 large nuclei, which may be an indirect sign of polyploidization. Well-developed organelles of the synthetic apparatus (ribosomes, polysomes, tubules of the granular endoplasmic reticulum) and mitochondria indicate active functional activity of these cells. This fact confirms the

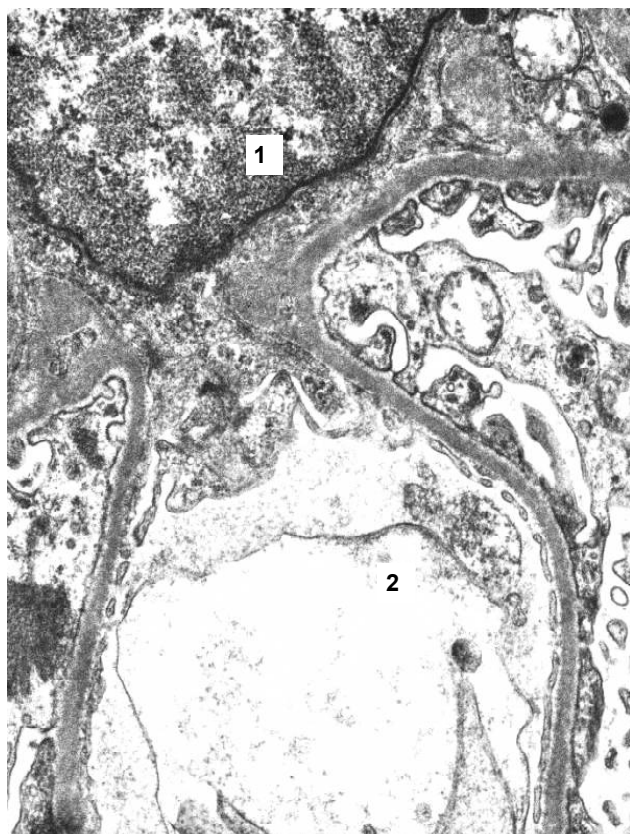


Fig. 2. Fragments of the renal corpuscles of rats' kidney after 22 days of MTBE use. Mesangial cells (1). Blisters of edema in the lumen of the capillaries (2). x8000.

increase in the amount of connective tissue in the glomeruli.

At the same time, mesangial cells with signs of dystrophic and destructive processes are observed. Such cells contain swollen tubules of the endoplasmic reticulum and mitochondria with local lysis of the matrix and cristae. On the surface of these cells there are microclasmatous growths and sequesters with edematous fluid, which protrude into the subendothelial layer, separating endothelial cells from the basement membrane. The ultrastructure of the proximal tubules after 22 days of MTBE action differs from that observed after 15 days. Most epitheliocytes have signs of varying degrees of dystrophic and destructive processes that extend to almost all cellular structures. Microvilli are usually delicate and often merge with each other or along the entire length, or in fragments. In some tubules their detachment from an apical surface is noted that leads to exposure of the last. Under the microvilli in the apical part of the cytoplasm there is a significant number of elongated or rounded secretory granules and primary lysosomes, most of which are electronically sealed. At the same time, the number of secondary and tertiary lysosomes in the central and basal parts of the cytoplasm is sharply reduced. Vacuoles with electronically transparent or finely dispersed, moderate electron density content are mainly observed.

After 60 days of MTBE use, the renal mass and their linear size become statistically significantly smaller than after 22 days of the experiment, but larger than in the control group. Microscopic examination draws attention to the appearance of sclerotic changes, which was not characteristic of rats of previous groups. Connective tissue growth is observed around the capsule of glomeruli, individual tubules and vessels. It should be noted that the glomeruli in this period of the experiment are significantly reduced in size, some atrophied and sclerosed, there is a small number of hypertrophied glomeruli. The variability of morphological changes in renal corpuscles attracts attention: structural manifestations of both hypertrophy and atrophy are revealed.

At the electron microscopic level, the lumens of the glomerular blood capillaries are usually dilated, often filled with shaped elements of blood, among which are identified cells at different stages of apoptosis. The endothelial cells of the blood capillaries of the glomeruli suffer significant damage: most of these cells are modified in the dark type with electronically compacted pycnomorphic nuclei. In the area around the nucleus, where most general-purpose organelles are located, it is difficult to distinguish individual structures. The peripheral parts of the cytoplasm of endothelial cells are unevenly thinned: significantly thinner

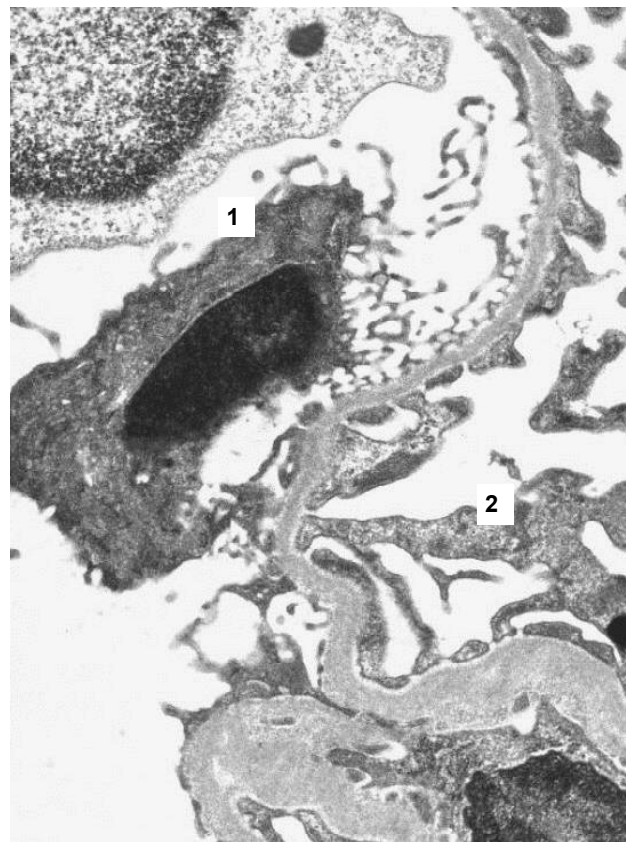


Fig. 3. Fragments of the renal corpuscles of rats' kidney after 60 days of MTBE use. Electronically sealed endothelial cells (1). Podocytes of moderate electron density (2). x10000.

areas of endotheliocytes are identified, in which the fenestrae disappear, and in other areas the size of the fenestrae reaches larger sizes. These processes occur against the background of marked destruction of other areas of endothelial cells, the cytoplasm of which acquires increased electron density, loses contact with the basement membrane and is desquamated (Fig. 3). The detected morphological changes indicate the development of apoptosis in the endotheliocytes of the blood capillaries of the glomeruli of the nephron. Deepened changes in the glomerular basement membrane. The areas where it connects endothelial cells and podocytes are thickened in places, locally destroyed in places. The mosaic structure of structural changes in podocytes is determined: some podocytes have a cytoplasm of moderate electron density, and in others the increase of electron density of cytoplasm in both trabeculae and cytopodia is determined. Dark podocytes usually had a pycnomorphic nucleus and destructively altered mitochondria.

Podocytes with medium electron density cytoplasm and electronically compacted cells are partially fragmented and desquamated into the lumen of the glomerular capsule. In some areas, cytopodia increase in size, acquire an irregular shape, thicken, are closely spaced, as a result of which the filtration gaps disappear between them. Filtration slits vary in size; most of them are covered with medium electron density filtration diaphragms, but in some places filtration diaphragms are not detected. Mesangial cells have structural signs of increased functional activity. In these cells large nuclei are defined, the nucleolemma of which forms numerous deep intussusception. The nuclei are quite electronically dense, heterochromatin in the form of different sized depths is concentrated near the inner leaf of the nucleolemma. In the nucleus there is a large electron-dense nucleolus. The cytoplasm of mesangial cells of high electron density with a significant number of ribosomes, polysomes, fibrous structures. Numerous mitochondria oval or elongated with well-developed cristae. A significant number of tubules of granular endoplasmic reticulum filled with medium electron density is determined. The phenomena of hyperplasia and hypertrophy of the Golgi complex are observed. Microfilaments are detected in the cytoplasm of mesangial cells. Activation of the biosynthetic activity of mesangial cells leads to an increase in the volume of the mesangial matrix and an increase in the content of fine-fiber structures in it, so that the processes of these cells appear to be walled up in the mesangium. Another consequence of significant processes of connective tissue biosynthesis is a significant thickening of basement membranes in areas of capillary bifurcations, as well as a large number of collagen fibers that accumulate around the renal corpuscles and in the interstitial space in the form of separate fibers or massive bundles. Epithelial cells of the proximal tubules have signs of dystrophic and destructive changes. Cells, as a rule, acquire an irregular shape, lose their inherent topography, the nuclei are often

displaced to the apical surface. Epitheliocytes with a cytoplasm of moderate electron density contain nuclei with uniformly distributed chromatin, but even in such cells the number of all organelles is reduced: both those involved in reabsorption (dense granules, lysosomes), and organelles of biosynthesis. Electronically compacted cells are observed, in which the number of organelles is also reduced, and the cytoplasm is filled with a fine substance, probably of protein origin. This substance can form local clusters or be located throughout the cytoplasm. The nuclei in such cells are pycnomorphic. Cells where this electronically dense substance displaces organelles and completely fills the entire cytoplasm lose their bond with each other, decrease in size, change shape, and take on the appearance of apoptotic cells.

Discussion

The study showed that the kidney is a very sensitive organ to MTBE. The dynamics of the experiment clearly revealed the stages of change and the dependence of their manifestations on the duration of action of this substance.

In the early stages of the experiment (3, 8 and 15 days) MTBE does not adversely affect kidney mass and its linear size. Increasing the duration of MTBE use (up to 22 days) causes changes in all the parameters studied, for example: the mass of the kidneys increases, respectively, increases its linear size compared to the control group. Long-term (60 days) action of MTBE led to a decrease in kidney mass and its linear size compared to the previous period of the experiment, but, nevertheless, the indicators did not reach the control values.

At the structural level, in the early stages (3 days) of MTBE action, reactive changes were detected, which were more clearly manifested in the structures of the filtration barrier.

Increasing the observation period to 8 days led to more pronounced changes, which extended to the epitheliocytes of the tubules, mainly proximal, which are characterized by the presence of cells at different stages of apoptosis.

After 15 days of the experiment, against the background of pronounced compensatory-adaptive changes, the development of dystrophic-destructive processes in both renal corpuscles and proximal tubules is noted. The main direction of changes in the cells of these parts of the nephron are changes in the dark type, ie apoptosis. Distal tubules, as in previous periods of the experiment, are more resistant to MTBE, they had no apoptotically altered epitheliocytes.

After 22 days of MTBE use, the renal ultrastructure differed from that observed after 15 days of MTBE. There are no endotheliocytes and podocytes in the renal corpuscles with signs of increased biosynthetic activity, which were distributed in the previous period. Changes in these cells take on a different direction: light cells predominate, while dark cells disappear, which is most likely due to their death. This is confirmed by the absence

of the smallest, atrophic bodies. Fusion and flattening of podocytes leads to a decrease in the number of diaphragm slits, which leads to a decrease in filtration processes. Increased functional activity of mesangial cells, which increased throughout the experiment, contributes to the deterioration of these processes due to increased connective tissue in the renal corpuscles. Electronically enlightened, swollen epitheliocytes are common in the proximal tubules, whereas dark cells are not observed. Dystrophic and destructive changes vary in severity in different epitheliocytes and extend to almost all cellular structures. The distal tubules do not show a significant deepening of ultrastructural changes, the greatest damage is suffered by basal membrane extrusion.

According to Y.I. Fedonyuk and V.V. Sikora [25] under the influence of heavy metal salts, the structural components of the renal nephron during the month of the experiment do not undergo significant changes. In the late stages of the experiment (60 days of MTBE action) compensatory-adaptive features apply only to a small number of cellular components of the nephron. Dystrophic and destructive changes, which vary in severity, predominate in all parts of the nephron. The most vulnerable to long-term action of MTBE are podocytes in the renal corpuscles and epitheliocytes of the proximal tubules. In cases where the damage becomes significant, there is atrophy of the renal corpuscles and devastation of the tubules, which leads to a decrease in their number. Increased activity throughout the experiment of cells that produce intercellular substance and, in particular, collagen fibers, leads to the development of renal fibrosis and the formation of severe renal sclerosis.

Similar dynamics of morphological changes of the gastric mucosa, cerebral cortex and thymus under the action of large doses of MTBE in similar terms of the experiment

is described in other studies [5, 6, 16]. The same dynamics of morphological changes in the structural components of the kidney under the influence of salts of heavy metals are determined after 2 months of the experiment [10]. According to the authors, the degree of pathological changes in the structural components of the kidney depends on the duration of heavy metals salts action on the kidneys. Under the influence of alcohol, similar structural lesions of nephron components are determined [22]. Thus, various toxic chemicals have a pronounced nephrotoxic effect, and the degree of damage to the structural components of the kidney is determined by the duration of toxic effects on the body.

In the future it is planned to study the histochemical changes in the mesangial cells of the renal corpuscle of the nephron in order to identify substances that are most actively synthesized by cells under the influence of different doses of MTBE. These studies will help determine which macromolecules accumulate in the glomerular basement membrane and in the mesangial matrix, which leads to their thickening and, thus, determines the disruption of filtration processes that occur through the filtration barrier of the renal nephron.

Conclusions

Thus, the study showed that MTBE has a pronounced nephrotoxic effect. MTBE adversely affects all structural components of the nephron, which leads to disorganization of the filtration and reabsorption apparatus of the kidney and the significant development of connective tissue, which causes sclerosis. The degree of damage to the structural components of the kidney by MTBE is determined by the duration of contact of the body with this toxic substance.

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МАКРО-МІКРОСКОПІЧНІ ЗМІНИ НИРОК ЩУРІВ ПІД ВПЛИВОМ МЕТИЛТРЕТБУТИЛОВОГО ЕФІРУ В РІЗНІ ТЕРМІНИ ЕКСПЕРИМЕНТУ

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Метилтретбутиловий ефір (МТБЕ) - це відносно новий антропогенний чинник забруднення навколишнього середовища. Вже відомо, що він є екологічно небезпечною речовиною, котра токсично впливає на організм людини. Метою дослідження є вивчення впливу МТБЕ на структурну організацію нирок експериментальних тварин. Дослідження виконано на білих безпородних щурах, що отримували 500 мг/кг МТБЕ в олійному розчині, який вводили внутрішньошлунково за допомогою зонда. Методами світлооптичної та електронної мікроскопії вивчали структурні зміни нирок на 3, 8, 15, 22 та 60-ту добу експерименту. У динаміці експерименту чітко визначена стадійність змін структурної організації нирки та залежність їх виразності від терміну дії цієї речовини. При короткотривалій дії (3, 8 та 15 доби експерименту) маса нирок та інші органометричні показники не відрізняються від аналогічних контрольної групи. На ранніх етапах дії МТБЕ виявляються реактивні зміни в структурах фільтраційного бар'єру (3 доби), які при збільшенні терміну спостережень (8 діб) поширюються і на епітеліоцити проксимальних канальців. Через 15 діб дії МТБЕ на тлі виразних компенсаторно-приспосувальних змін відмічається розвиток дистрофічно-деструктивних процесів у ниркових тільцях і в проксимальних канальцях. В мезенгіальних клітинах виявляються структурні ознаки посиленої синтетичної активності. На 22 добу експерименту морфометричні показники маси та лінійних розмірів нирок збільшуються порівняно з контрольною групою. В нефронах визначаються структурні ознаки як процесів атрофії, так і гіпертрофії. Канальцевий апарат нирок зазнає прогресуючих змін у порівнянні із попереднім терміном експерименту. Довготривала дія МТБЕ (60 діб) викликає подальше поглиблення дистрофічних та деструктивних змін в усіх відділах нефрону, які варіюють за проявами. Визначено, що МТБЕ негативно впливає на всі структурні компоненти нефрону, що призводить до дезорганізації фільтраційного та реабсорбційного апарату нирки і значного розвитку сполучної тканини, що обумовлює явища склерозу. Доведено, метилтретбутиловий ефір має виражений нефротоксичний ефект і ступінь ураження структурних компонентів нирки визначається тривалістю контакту організму з цією токсичною речовиною.

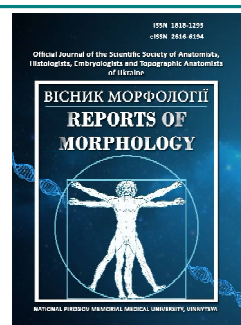
Ключові слова: нирки, метилтретбутиловий ефір, нефрон.



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Sex-age features of the prevalence and structure of heart rhythm disorders in the patients with severe Covid-infection

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There is growing evidence that arrhythmias are a major complication of COVID-19 infection. Inflammation of the myocardium, cytokine storm, hypoxia, changes of electrolyte levels, coronary vasospasm, microclots may be factors that contribute to the development of arrhythmias and changes of the ECG. The aim of the study was to determine the sex and age characteristics of the prevalence and structure of cardiac arrhythmias in patients with severe COVID infection. In the study were examined 133 patients with severe COVID-19 infection, who were in the intensive care unit of the city clinical hospital №1 in Vinnytsya at the period from April to December 2020 and as a result of treatment with improved health were discharged from the hospital. Statistical processing of the obtained data was performed using the statistical software package SPSS 12.0 for Windows using parametric and non-parametric methods of statistical analysis. It was found that the most common comorbid pathologies of the patients with severe COVID-19 were - arterial hypertension 90 (67.7%), coronary heart disease 91 (68.4%), chronic heart failure 65 (48.9%), obesity 47 (35.3%) and diabetes mellitus 32 (24.1%). It was found that the prevalence of cardiac arrhythmias in patients with severe COVID-19 was 86 (64.7%) patients. Among all arrhythmias, tachyarrhythmias predominated - 68 (51.1%), in the structure of which sinus tachycardia was the most common in 45 (33.8%) and atrial fibrillation (AF) - in 19 (14.3%) patients, respectively. In turn, sinus tachycardia was significantly more common among women. The prevalence of bradyarrhythmias was 60 (45.1%), in the structure of which were dominated sinus bradycardia 16 (12%) and blockade of the right branch of the His bundle 30 (22.6%). Among men, there was a tendency to the predominance of complete left bundle branch block. The prevalence of ischemic changes and/or repolarization disorders according to the ECG was 119 (89.5%), in the structure of which were dominated the presence of inversion of the T wave 91 (68.4%) and depression of the ST segment 54 (40.6%). The mean age of the patients with ischemic ECG changes was significantly higher compared to patients without signs of repolarization disorders. Among men, there was a predominance of pancreatic hypertrophy and a tendency to predominance of left ventricular hypertrophy.

Keywords: COVID, heart rhythm disorders, arrhythmia, atrial fibrillation, premature beat, heart block, arterial hypertension, coronary heart disease, chronic heart failure.

Introduction

The experience we gained during the epidemic of COVID-19 infection showed that a significant proportion of patients have various cardiovascular complications. Namely, myocarditis, heart failure and acute coronary syndrome resulting from coronary artery thrombosis or rupture of atherosclerotic plaques as a complication of COVID infection.

There is growing evidence that arrhythmias are also a major complication. Inflammation of the myocardium

caused by a viral infection leads to electrophysiological and structural remodeling as a possible mechanism of arrhythmias [4]. Also, hypoxia and electrolyte changes during the acute phase of COVID infection have often been reported in patients with severe disease, which are known to contribute to the development of acute arrhythmias [20].

A recent study reported that about 7% of patients with COVID infection complain of palpitations [22]. According to a report by Chinese researchers D. Wang and co-authors

(2020), cardiac arrhythmias were reported in 16.7% of hospitalized and 44.4% of patients in the COVID-19 intensive care unit [29]. In the analysis of 115 patients (69 of them hospitalized in the intensive care unit and 46 patients in other departments), it was found that paroxysms of supraventricular tachyarrhythmias, namely atrial fibrillation (AF), flutter and atrial tachycardia (PT), were observed in 19 patients (16.5%) among all hospitalized and all of them were in the intensive care unit (27.5%) [10].

According to a survey of 1,100 electrophysiologists from 76 countries, AF was the most common tachyarrhythmia, and sinus bradycardia and complete atrioventricular block were the most common bradyarrhythmias in patients with COVID-19 infection [13]. The mechanisms that can cause AF in these patients may be due to systemic infection, direct viral cardiomyocyte involvement, hypoxemia and susceptibility due to old age, comorbidities, and overactive sympathetic nervous system [27].

Another study found that among 187 hospitalized patients, 13 (7%) had ventricular tachyarrhythmias during hospitalization. In addition, malignant arrhythmias, including ventricular tachycardia/ventricular fibrillation, were more common in patients with elevated T-troponin levels [14]. Ventricular arrhythmias and torsade de pointes have also been reported due to QT prolonging drugs, especially azithromycin and hydroxychloroquine [9].

Therefore, the exact effect of COVID-19 infection on the development of arrhythmias in asymptomatic, mildly ill, critically ill and recovered patients is unknown [20]. There are currently no studies aimed directly at studying arrhythmias in patients with COVID-19. Of particular interest to us, both from a practical and scientific point of view, is the study of the prevalence and features of cardiac arrhythmias among patients with COVID-19 infection among the population of Ukraine.

The aim of the study was to establish the sex and age characteristics of the prevalence and structure of cardiac arrhythmias in patients with severe COVID infection.

Materials and methods

133 patients who were in the intensive care unit of the city clinical hospital №1 Vinnytsia in the period from April to December 2020 were examined and were discharged from the hospital with improved health.

Among them there were 65 (48.9%) men and 68 (51.1%) women ($\chi^2=0.068$, $p=0.79$). The age of patients ranged from 24 to 90 years, averaging 61.38 ± 12.96 years. Age gradation showed that the group under the age of 45 included 13 (9.8%) patients, 45-59 years - 46 (34.6%), 60-74 years - 56 (42.1%), 75-90 years - 18 (13.5%) patients. The age structure was dominated by middle-aged and elderly patients ($p=0.001$).

The total length of stay in hospital was 18.90 ± 9.12 days. The length of stay in the intensive care unit varied from 1 to 37 days and averaged 8.320 ± 6.919 days.

The polymerase chain reaction was performed in all

Table 1. The structure of comorbidity in patients with severe COVID-infection.

Comorbidity	Proportion of patients (n=133)
Hypertension (n=90)	67.7%
IHD (n=91)	68.4%
CHF (n=113)	85.0%
Combination of Hypertension and IHD (n=79)	59.4%
DM (n=32)	24.1%
Coronary artery bypass grafting (n=2)	1.5%
Prosthetics of valves (n=2)	1.5%
Rheumatic heart disease (n=1)	0.8%
Kidney disease (n=13)	9.8%
Cancer (n=8)	6.0%
Gastrointestinal diseases (n=17)	12.8%
COPD/Asthma (n=9)	6.8%
Acute cerebrovascular accident in the anamnesis (n=11)	8.3%
Thyroid disease (n=9)	6.8%
Multiple sclerosis (n=1)	0.8%
Anxiety and depressive disorder (n=1)	0.8%
Parkinson's disease (n=1)	0.8%
Raynaud's syndrome (n=2)	1.5%
Metabolic cardiomyopathy (n=2)	1.5%
Flu (n=2)	1.5%
Exacerbation of erysipelas (n=1)	0.8%
Gout (n=2)	1.5%
Diseases of the musculoskeletal system (n=5)	3.8%

patients. A positive result was in 50 (37.6%) patients, negative - in 83 (62.4%). In turn, rapid testing to determine the presence of the virus was not performed in 44 (33.1%) cases, a positive result was - in 27 (20.3%), a negative result - in 62 (46.6%) patients. Enzyme-linked immunosorbent assay for antibodies to Sars-Cov-2 virus was not performed in 20 (15.0%) patients, positive test result was - in 97 (72.9%), negative result - in 16 (12.0%) patients.

Chest computed tomography (CT) was performed on 69 (51.9%) patients. According to CT OTC - unilateral lesions of the lung parenchyma were - in 4 (9.0%), bilateral - in 61 (81.0%) and 2 patients showed changes in the type of bronchitis.

The diagnosis of COVID infection was established: according to the combination of a specific clinical picture and a positive polymerase chain reaction for the presence of Sars-Cov-2 virus was in 50 (37.6%) patients; increase in IgM titers to Sars-Cov-2 virus in 75 (56.4%) patients; 6 (4.5%) patients tested positive for the virus; in the presence of specific changes in the lung parenchyma according to CT - in 2 (1.5%) patients.

Analysis of patients' temperature showed that normal

body temperature was in 6 (4.5%), subfebrile - 59 (44.4%), moderately high - 56 (42.1%), high - 11 (8.3%), hyperpyretic - 1 (0.8%) patient. The vast majority of patients had fever of moderately high and high levels ($p=0.001$).

The most common comorbidities in the examined patients (Table 1) were Hypertension, IHD, CHF, obesity and DM. Hypertension was diagnosed in 90 (67.7%) individuals, including 45 (50.0%) women and 45 (50.0%) men ($\chi^2=0.142$, $p=0.7$). Analyzing the severity of Hypertension, it was found that 7 (5.3%) patients had stage 1, 72 (54.1%) had stage 2, 15 (11.3%) had stage 3, and 8 (6%) - 1 degree, in 74 (55.6%) - 2 degree, in 12 (9%) - 3 degree Hypertension, respectively. The course of Hypertension in 32 (24.1%) patients was uncontrolled, in 101 (75.9%) - controlled, respectively ($\chi^2=5.14$, $p=0.02$). In turn, the uncontrolled course of Hypertension was observed in 14 (10.5%) women and 18 (13.5%) men, respectively ($\chi^2=0.918$, $p=0.338$). The risk of cardiovascular complications in 4 (3.0%) - low, 33 (24.8%) - moderate, in 50 (37.6%) - high and in 46 (34.6%) patients - very high.

Among this array of patients, 91 (68.4%) patients had IHD, namely angina pectoris - 7 (5.3%), postinfarction cardiosclerosis - 8 (6.0%), diffuse cardiosclerosis - 76 (57.1%) patients, respectively. Also, a significant proportion - 79 (59.4%) patients had a combination of Hypertension and IHD.

In turn, 113 (85.0%) patients had signs of CHF, among them - stage I CHF was observed in 65 (48.9%), stage IIA - in 45 (33.8%), stage IIB - in 3 (2.3%) patients, respectively.

Obesity affected 47 (35.3%) patients, including 26 (19.5%) - stage 1, 11 (8.3%) - stage 2, 12 (9.0%) - stage 3 obesity, respectively. 32 (24.1%) patients had T2D.

Among the acute complications that occurred during the stay of patients in hospital: acute myocardial infarction - in 2 (1.5%), acute cerebrovascular accident (ACA) - in 3 (2.3%), acute myocarditis - in 1 (0.8%) %, acute thrombophlebitis - in 15 (11.3%), acute heart failure - in 4 (3.0%), verified pulmonary embolism - in 3 (2.3%), sepsis/infectious-toxic shock - in 1 (0.8%) patient, respectively.

Statistical processing of the obtained data was performed on a personal computer using the statistical software package SPSS 12.0 for Windows. Quantitative data (with a normal distribution of characteristics) are presented in the form ($M\pm\sigma$), where M is the average value of the sample, and σ is the standard deviation. The reliability of the difference in quantitative values was calculated by Student's criterion, the percentage - χ^2 [25].

Results

Assessment of the severity of COVID infection according to OTC CT (Fig. 1) showed that the absence of specific changes (CT - 0) in the lungs was 2 (2.9%), slight changes according to the type of "frosted glass" with a lesion <25% lung parenchyma (CT-1) (average $17.00\pm4.47\%$) - in 5 (7.3%), moderate changes (CT-2) with a lesion of 25-50% (average $37.56\pm7.48\%$) - in 16 (23.2%) patients, severe

changes (CT-3) with a lesion of 50-75% (average $62.40\pm8.08\%$) - in 25 (36.2%), critically severe impressions (CT-4) involving >75% (average 87.62 ± 10.56) - in 21 (30.4%) patients, respectively. This indicated a predominance of patients with moderate, severe and critically severe lung parenchyma (23.2%, 36.2% and 30.4% vs. 2.9% and 7.3%, $p=0.001$).

Also, according to the CO-RADS classification, which is standardized for patients with suspected COVID-19, the typical changes for CO-RADS 3 (medium risk of COVID) were in 1 (1.5%) patient, CO-RADS 4 (high risk COVID) - in 10 (14.5%) patients, CO-RADS 5 (very high risk of COVID) - in 58 (84.0%) patients. Thus, most of the examined subjects had diffuse areas of turbidity of the "frosted glass" type and compaction of the lung parenchyma (CO-RADS 5) (84.0% vs. 14.5% and 1.5%, $p=0.001$).

The vast majority of patients had bilateral lung damage (97% vs. 3%, $p=0.0001$). Pulmonary insufficiency (Fig. 2) of the first degree was in 10 (7.5%), second degree - in 41 (30.8%), third degree - in 82 (61.7%) patients, respectively. Acute respiratory distress syndrome was found in 6 (4.5%) cases. Analysis of the severity of pulmonary insufficiency showed that the vast majority of patients had severe pulmonary insufficiency (61.7% vs. 30.8% and 7.5%, $p = 0.001$).

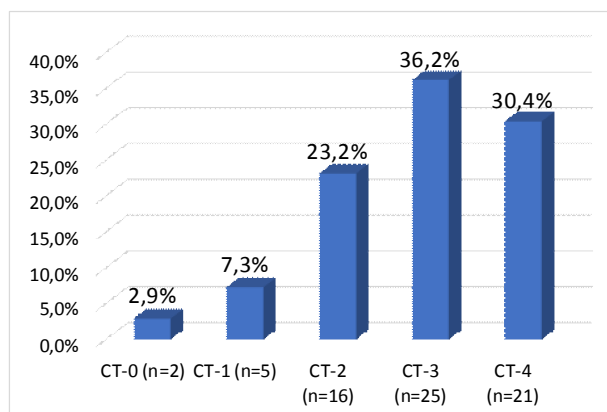


Fig. 1. Comparative graphs of the severity of lung parenchymal lesions according to chest computed tomography (CT).

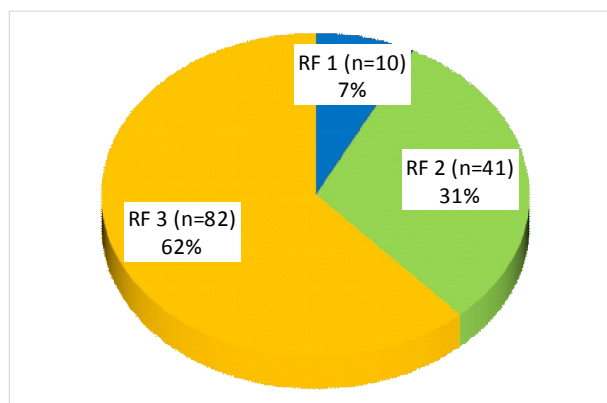


Fig. 2. The proportion of severity of respiratory failure (RF) in the patients with severe COVID-19 infection.

Oxygen therapy using a face mask or nasal cannula was received by 76 (57.1%) patients, by non-invasive CPAP therapy 56 (42.1%) patients. No patient was switched to invasive oxygen support and 1 patient did not need oxygen support.

Analyzing laboratory parameters, it was found that anemia was present in 44 (33.1%) patients. In turn, a decrease in glomerular filtration rate (GFR) less than 60 ml/min/1.72 m² - in 82 (61.7%) patients (61.7% vs. 37.6%, p=0.005). GFR ranged from 9 to 114 ml/min/1.72 m² (according to the formula CKD-EPI) and averaged 54.11±21.54 ml/min/1.72 m².

The level of C-reactive protein (n=71), procalcitonin (n=65), interleukin - 6 (n=68), D-dimer (n=57) in patients with severe COVID during the period of stay in the intensive care unit was also analyzed. therapy.

According to the methods of variation statistics, it was

obtained that the minimum and maximum levels of C-reactive protein were - 8.030 and 406.2 mg/l, respectively. The average value of the indicator is 135.8±86.4 mg/l, the median is 124.0 mg/l. Thus, the relatively low level (RLL) of C-reactive protein for this sample was defined as a value less than 25 percentile (<68 mg/l) - 18 (13.5%), the intermediate level of C-reactive protein as a value between 25 and 75 percentile (from 69 to 182 mg/l) - 35 (26.3%) and relatively high level (RHL) of C-reactive protein - > 75 percentile, respectively (>183 mg/l) - 18 (13.5%) patients, respectively.

The minimum and maximum levels of procalcitonin were 0.01 and 7.57 ng/ml, respectively. The average value of the indicator is 0.429±1.044 ng/ml, the median is 0.13 ng/ml. Thus, the RLL of procalcitonin for this sample was defined as a value less than 25 percentile (<0.07 ng/ml) - 20 (15.0%), the intermediate level of procalcitonin as a

Table 2. Comparative data of patients' sex and age with severe COVID infection.

Parameters	Men (n=65)	Women (n=68)	Total (n=133)	p value men/women
Age	60.55±13.38	62.18±12.6	61.38±12.96	0.47
Term of hospitalization	18.86±7.14	18.94±10.75	18.90±9.12	0.96
The period of stay in intensive care	7.800±5.133	8.820±8.284	8.320±6.919	0.4
Hypertension	45 (69.2%)	45 (66.2%)	90 (67.7%)	0.7
IHD	47 (72.3%)	44 (64.7%)	91 (68.4%)	0.34
Combination of Hypertension and IHD	38 (58.5%)	41 (60.3%)	79 (59.4%)	0.83
ACA	5 (7.7%)	6 (8.8%)	11 (8.3%)	0.81
CHF	53 (81.5%)	60 (88.2%)	113 (85%)	0.28
T2D	15 (23.1%)	17 (25%)	32 (24.1%)	0.79
Hyperglycemia	10 (15.4%)	19 (27.9%)	29 (21.8%)	0.08
Reduction of GFR <60 ml/min/1.73 m ²	34 (52.3%)	48 (70.5%)	82 (61.7%)	0.02
Average GFR	59.71±22.8	48.75±18.93	54.11±21.54	0.003
Anemia	20 (30.8%)	24 (35.3%)	44 (33.1%)	0.58
Obesity	20 (30.8%)	27 (39.7%)	47 (35.3%)	0.28
Thyroid disease	2 (3.1%)	7 (10.3%)	9 (6.7%)	0.09
COPD/Asthma	5 (7.7%)	4 (5.9%)	9 (6.7%)	0.67
Cancer	3 (4.6%)	5 (7.4%)	8 (6%)	0.5
Gastrointestinal diseases	6 (9.2%)	11 (16.2%)	17 (12.8%)	0.23
CRP	142.4±83.5	127.7±90.5	135.8±86.4	0.48
Procalcitonin	0.247±0.236	0.629±1.478	0.429±1.044	0.14
Interleukin-6	207.9±694.4	219.0±797.9	212.8±736.1	0.95
D-dimer	0.975±1.479	1.430±1.995	1.207±1.760	0.33
Increased fibrinogen (n=112)	46 (82.1%)	54 (96.4%)	100 (89.2%)	0.01
Na level	139.5±5.2	138.9±4.4	139.2±4.8	0.52
K level	3.968±0.912	4.122±0.905	4.045±0.907	0.39
The degree of severity according to CT	2.920±0.996	2.760±1.091	2.840±1.038	0.53
Accumulation of fluid in the pleural cavity	10 (15.4%)	4 (5.9%)	14 (10.5%)	0.07

Notes: the reliability of the difference in quantitative values is calculated by Student's criterion. percent - according to criterion χ^2 .

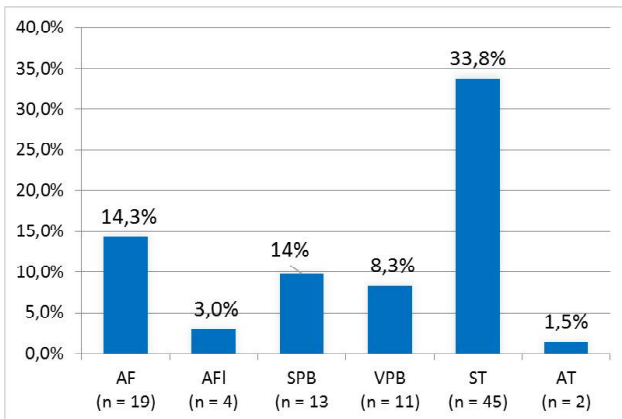


Fig. 3. Comparative data of the proportion of the patients with tachyarrhythmias at severe COVID-19 infection. AF - atrial fibrillation, AFI - atrial flutter, SPB - supraventricular premature beats, VPB - ventricular premature beats, ST - sinus tachycardia, AT - atrial tachycardia.

value between 25 and 75 percentile (from 0.08 to 0.34 ng/ml) - 27 (20.3%) patients and RHL of procalcitonin - > 75 percentile, respectively (>0.35 ng/ml) - 18 (13.5%) patients, respectively.

In turn, the minimum and maximum levels of interleukin-6 are 2.95 and 4425 pg/ml, respectively. The average value of the indicator is 212.8±736.1 pg/ml, the median is 54.22 pg/ml. Thus, the RLL of interleukin-6 for this sample was defined as a value less than 25 percentile (<23 pg/ml) - 18 (13.5%), the intermediate level as a value between 25 and 75 percentile (from 24 to 109 pg/ml) - 33 (24.8%) and RHL - > 75 percentile, respectively (>110 pg/ml) - 17 (12.8%) patients, respectively.

The minimum and maximum levels of D-dimer were 0.14 and 8.31 µg, respectively. The mean value was 1.210±1.760 µg, and the median was 0.54 µg. Thus, the RLL of the D-dimer was defined as a value less than 25 percentile (<0.3 µg) - 18 (13.5%), the intermediate level as a value between 25 and 75 percentile (0.4 to 1.0 µg) - 20 (15.0%) and RHL - > 75 percentile, respectively (>1.1 µg) - 19 (14.3%) patients, respectively.

Analyzing the sex and age characteristics of the examined patients with severe COVID infection (Table 2) it was found that men and women did not differ in average age, length of hospitalization and period of stay in the intensive care unit, the structure of comorbid pathology, the results of most additional methods. However, there was a tendency that the proportion of patients with thyroid disease (10.3% vs. 3.1%, p=0.09) and hyperglycemia among women was higher (27.9% vs. 15.4%, p=0.08). Also, females had a lower average level of glomerular filtration rate (48.75 vs. 59.71, p=0.003) and the proportion of patients with GFR <60 ml/min/1.73 m² was significantly higher compared to men (70.5% against 52.3%, p=0.02). Among the male, according to CT/radiography OTC there was a tendency to the predominance of fluid accumulation in the pleural cavity (15.4% vs. 5.9%, p=0.07).

The frequency and structure of cardiac arrhythmias among patients with severe COVID infection were also analyzed according to a standard 12-lead electrocardiogram.

During the period of inpatient treatment in the vast majority of 86 (64.7%) patients observed the presence of arrhythmias (64.7% vs. 34.6%, p=0.001). Among them, 42 (64.6%) men and 44 (64.7%) women, p=0.58. In turn, the average length of stay in the intensive care unit in patients with arrhythmias (9.43 vs. 6.3 days, p=0.02) and the total length of hospitalization (20.22 vs. 16.49, p=0.01) were significantly longer compared to the group of individuals without arrhythmias. It should be noted that the prevalence of arrhythmias did not depend on the severity of lung lesions according to CT OTC, the severity of pulmonary insufficiency and the clinical group of community-acquired pneumonia among patients with severe COVID-19.

68 (51.1%) patients with tachyarrhythmia took the leading position in the structure of disorders (Fig. 3). Among them, a number of patients had a combination of different heart rhythm disorders. Thus, AF was observed in 19 (14.3%) patients, namely in 7 (5.3%) paroxysmal, in 3 (2.3%) - persistent and in 9 (6.8%) patients - permanent form AF, respectively (Table 3). Among patients with acute AF paroxysms, 6 (60.0%) patients underwent drug cardioversion and 4 (40.0%) patients had spontaneous recovery of sinus rhythm within the first 48 hours after the onset of arrhythmia paroxysm. The CHA2DS2VASc score ranged from 2 to 5 and averaged 3.26±0.99, indicating that all AF patients had a high risk of thromboembolic complications.

In turn, Atrial fibrillation was registered in 4 (3.0%), Atrial tachycardia in 2 (1.5%), sinus tachycardia (ST) in 45 (33.8%) patients. Frequent supraventricular arrhythmias - in 13 (9.8%), ventricular arrhythmias (VA) - in 11 (8.3%), among them - 1st grade according to Lown - in 7 (5.3%), 2nd grade - in 3 (2.3%), 4 b grade - in 1 (0.8%) patient.

After analyzing the sex and age characteristics of patients (Table 4) with tachyarrhythmias, it was found that the average age of patients with and without tachyarrhythmias did not differ (61.35 vs. 61.42, p=0.97). In turn, there was no significant sex difference in the structure of arrhythmias, however, among women there was a tendency to a predominance of sinus tachycardia (41.2% vs. 26.2%, p=0.06).

Patients in whom, according to the ECG, different types of bradyarrhythmias were recorded 60 (45.1%), in some cases had a combination of different conduction disorders.

Table 3. Distribution of patients by forms of atrial fibrillation.

AF characteristics	Proportion of patients (n=19)
<i>Forms of AF</i>	
Paroxysmal (n=7)	5.3%
Persistent (n=3)	2.3%
Constant (n=9)	6.8%

Table 4. Sex and age features of tachyarrhythmias structure in patients with severe COVID infection.

Rhythm disorders	Men (n=65)	Women (n=68)	p value
Tachyarrhythmias total (n=68)	33 (50.8%)	35 (51.5%)	=0.93
AF (n=19)	10 (15.4%)	9 (13.2%)	=0.72
Sinus tachycardia (n=45)	17 (26.2%)	28 (41.2%)	=0.06
Atrial fibrillation (n=4)	3 (4.6%)	1 (1.5%)	=0.28
Atrial tachycardia (n=2)	2 (3.1%)	0 (0%)	=0.14
Supraventricular arrhythmia (n=13)	7 (10.8%)	6 (8.8%)	=0.7
Ventricular arrhythmia (n=11)	7 (10.8%)	4 (5.9%)	=0.3

Notes: the reliability of the percentage difference is calculated according to criterion χ^2 .

Table 5. Sex and age features of bradyarrhythmias structure in patients with severe COVID infection.

Rhythm disorders	Men (n=65)	Women (n=68)	p value
Bradyarrhythmias total (n=60)	32 (49.2%)	28 (41.2%)	=0.35
Sinus bradycardia (n=16)	9 (13.8%)	7 (10.3%)	=0.52
LBBB (n=3)	3 (4.6%)	0 (0%)	=0.07
LAFB (n=11)	5 (7.7%)	6 (8.8%)	=0.81
LPFB (n=4)	1 (1.5%)	3 (4.4%)	=0.33
RBBB (n=30)	17 (26.2%)	13 (19.1%)	=0.33
First-degree AV block (n= 14)	7 (10.8%)	7 (10.3%)	=0.92
Second-degree AV block (n=1)	1 (1.5%)	0 (0%)	=0.3

Notes: the reliability of the percentage difference is calculated according to criterion χ^2 .

Table 6. Sex and age features of ischemic changes structure and repolarization disorders in patients with severe COVID infection.

Rhythm disorders	Men (n=65)	Women (n=68)	p value
Negative T (n=91)	44 (67.7%)	47 (69.1%)	=0.86
Early repolarization (n=14)	5 (7.7%)	9 (13.2%)	=0.29
Elevation of the ST segment (n=6)	3 (4.6%)	3 (4.4%)	=0.95
Depression of the ST segment (n=54)	25 (38.5%)	29 (42.6%)	=0.62
Pathological Q wave (n=9)	5 (7.7%)	4 (5.9%)	=0.67
LV overload (n=27)	13 (20.0%)	14 (20.5%)	=0.93
LV hypertrophy (n=45)	27 (41.5%)	18 (26.5%)	=0.06
RV overload (n=8)	5 (7.7%)	3 (4.4%)	=0.42
RV hypertrophy (n=12)	9 (13.8%)	3 (4.4%)	=0.05
SIQIII sign	5 (7.7%)	2 (2.9%)	=0.22
QT prolongation	1 (1.5%)	5 (7.35%)	=0.1
P-pulmonale (n=6)	3 (4.6%)	3 (4.4%)	=0.95
P-mitrale (n=7)	4 (6.2%)	3 (4.4%)	=0.65
Delta wave (n=1)	0 (0%)	1 (1.5%)	=0.32

Notes: the reliability of the percentage difference is calculated according to criterion χ^2 .

Among the blockades were registered: sinus bradycardia in 16 (12.0%), left bundle branch block (LBBB) - in 3 (2.3%), left anterior fascicular block (LAFB) - in 11 (8.3%), left posterior fascicular block (LPFB) - in 4 (3.0%), right bundle branch block (RBBB) - in 30 (22.6%), First-degree AV block - in 14 (10.5%), Second-degree AV block - in 1 (0.8%) patient, respectively.

Analyzing the sex and age characteristics of patients with conduction disorders (Table 5), it was found that the average age of patients with blockade was higher compared with patients without bradyarrhythmias (64.05 vs. 59.19, $p=0.03$). In the structure of bradyarrhythmias among men there was a tendency to predominance of LBBB, compared with women (4.6% vs. 0%, $p=0.07$).

The results of the ECG analysis showed that the majority of patients (119 people, which was 89.5%) - had various ischemic changes and/or disorders of myocardial repolarization. Thus, inversion of the T wave was registered in 91 (68.4%), signs of early ventricular repolarization in 14 (10.5%), elevation of the ST segment in 6 (4.5%), depression of the ST segment in 54 (40.6%), pathological Q wave - in 9 (6.8%). In turn, the signs of left ventricular overload were determined - in 27 (20.3%), right ventricle - in 8 (6.0%), the sign of SIQIII - in 7 (5.3%), QT prolongation - in 6 (4.5%) of patients. Also signs of left ventricular hypertrophy - 45 (33.8%), signs of right ventricular hypertrophy - in 12 (9.0%), the presence of p-pulmonale - 6 (4.5%), p-mitrale - 7 (5.3%) patients, respectively.

Analyzing the sex and age characteristics of patients with ischemic changes/repolarization disorders (Table 6), it was found that the average age of patients with ischemic changes on the ECG was significantly higher compared to patients without signs of repolarization disorders (62.13 vs. 55.0, $p=0.05$). In the structure of signs of repolarization disorders among men there was a predominance of right ventricular hypertrophy (13.8% vs. 4.4%, $p=0.05$) and a tendency to predominance of left ventricular hypertrophy (41.5% vs. 26.5%, $p=0.06$).

Discussion

Our study found that the most common comorbidities in patients with severe COVID-19 were Hypertension - 67.7%, IHD - 68.4%, CHF - 48.9%, obesity - 35.3% and diabetes - in 24.1% of patients, respectively. Our data are in some way consistent with the results of studies by other scientists, which analyzed the structure of comorbid pathology among individuals with severe COVID-19.

Thus, among the 5,700 patients with COVID-19 who were hospitalized in 12 New York hospitals between March 1 and April 4, 2020, the most common comorbidities were Hypertension (56.6%), obesity (41.7%), and diabetes (33.8%). In turn, 30.7% of patients had fever, 17.3% had a respiratory rate of more than 24 breaths/min, and 27.8% required oxygen therapy [26]. In a meta-analysis of 25 studies (4881 patients) related to the study of COVID-19 found that the most common comorbidity among patients

with severe disease was Hypertension (33.4%), in second place - diabetes (14.4%) [31].

We found that the prevalence of cardiac arrhythmias in patients with severe COVID-19 is 67.7%. Some literature sources also describe data on the analysis of the prevalence and structure of arrhythmias in patients with severe COVID-19. Thus, in a report by Chinese scientists Wang D. et al. (2020) cardiac arrhythmias were reported in 44.4% of patients in the COVID-19 intensive care unit [29]. A meta-analysis of 5 studies (1553 patients) with COVID-19 was also performed. Among 349 (22.47%) patients with severe disease, in 105 (30.09%) developed various arrhythmias [32].

In the course of our study it was found that among all arrhythmias, tachyarrhythmias predominate - 51.1%, in the structure of which the most common sinus tachycardia - 48% and AF - 14.3% of patients, respectively. In turn, sinus tachycardia was significantly more common among women. The prevalence of bradyarrhythmias was 45.1%, the structure of which was dominated by sinus bradycardia - in 12% and RBBB - in 22.6%. Among men, there was a tendency for LBBB to predominate.

According to the available literature, among patients with COVID-19 AF was detected in 19-21% of all cases [13, 17]. One study reported the prevalence of AF in 36% of patients with cardiovascular disease, with AF observed in 42% of patients who did not survive [17]. In a small report, up to 75% of hospitalized geriatric patients with COVID-19 had a history of AF in the past [12]. The latest statistics from the COVID-19 working group of the Italian National Institutes of Health showed that 24.5% of the 355 patients who died of COVID-19 (mean age 79.5 years, 70% of men) had a history of AF before infection with SARS-CoV-2 [23]. Reliable data on the first-onset AF in patients with COVID-19 are limited. Based on reports of clinical cases and small studies, the prevalence of the first AF paroxysm ranges from 3.6% to 6.7% in patients with COVID-19 [3, 7, 8, 28]. Both sinus tachycardia and AF are independent predictors of disease severity, myocardial injury, and poor outcomes in COVID-19 [30].

Also, in a single-center US study of 700 patients with COVID-19 (11% of whom were in the intensive care unit), AF was observed in 25 patients, significant bradyarrhythmias in 9 patients, and unstable VT in 10 patients, however, no patient had persistent monomorphic ventricular tachycardia, ventricular fibrillation, or complete heart block [7].

In a study by D. Kir et al (2020), a patient with COVID-19 infection who had unchanged echocardiography and cardiac biomarkers developed bradycardia and intermittent high-grade atrioventricular block [19]. G. Peigh and co-authors (2020) reported sinus node dysfunction in two cases of COVID-19. Sinus bradycardia with subsequent episodes of accelerated idioventricular rhythm was observed in these patients [24]. In a clinical case, a patient with COVID-19 is described who suffered from grade 1

atrioventricular block but during hospitalization the rhythm switched to grade 2 atrioventricular block type Mobitz-1 followed by the development of complete atrioventricular block [16]. In other cases, elderly patients with multiple cardiac risk factors who have high-grade atrioventricular block and/or intraventricular conduction block are described [2, 18].

Transient sinus bradycardia is a possible manifestation of COVID-19 and is important for close monitoring. The etiology can be multifactorial, namely hypoxia, inflammatory cardiomyocyte damage, and drug response may be triggers for sinus bradycardia. High levels of pro-inflammatory cytokines can directly affect the sinoatrial node, contributing to the development of bradycardia. This may be a harbinger of the onset of a serious cytokine storm. In turn, sinus bradycardia, nodal rhythm, idioventricular rhythm occurred immediately before cardiac arrest - therefore, the development of bradyarrhythmias in a patient with severe COVID-19 is a marker of the risk of approaching cardiovascular collapse [1].

Of undoubted scientific interest was a fragment of the study devoted to the study of the prevalence of ischemic changes and/or repolarization disorders. Such changes on the electrocardiogram were found in 89.5% of patients, the structure of repolarization disorders was dominated by the presence of inversion of the T wave (68.4%) and depression of the ST segment (40.6%). In turn, the mean age of patients with ischemic changes on the electrocardiogram was significantly higher compared to patients without signs of repolarization disorders. Among men, there was a predominance of right ventricular hypertrophy and a tendency to predominate left ventricular hypertrophy.

It is known that COVID-19 infection, associated with myocardial damage, can cause ST segment deviation (elevation or depression), inversion of the T wave and the appearance of pathological Q wave [11, 15]. One study found that ST-segment and T-wave changes were the most common abnormalities in patients requiring hospitalization in the intensive care unit and occurred in 40% of patients [21]. Another study noted that nonspecific repolarization changes, including ST-segment and T-wave abnormalities, were found in 41% of patients. These changes have been reported as a result of myocardial damage and are associated with poor prognosis, including an increased need for hospitalization, more frequent mechanical ventilation support, and increased mortality [6, 30]. Another study involved 219 patients hospitalized through COVID-19 from April 15 to May 5, 2020. Patients were divided into two groups according to the severity of COVID-19 infection: severe (n=95) and mild (n=124). ST-segment depression (28% vs. 14%), T-wave inversion (29% vs. 16%), ST-T changes (36% vs. 21%), and the presence of fragmented QRS (fQRS) (17% vs. 7%) were more common. in the group with severe disease, compared with patients with mild COVID-19 [5].

Thus, the literature data, in some way, confirm the results obtained in our study. In general, it can be concluded that COVID-19 infection adversely affects the cardiovascular system and leads to changes in the ECG, which may be due to cytokine storm, hypoxia, changes in electrolytes, coronary spasm, microthrombi, and direct endothelial or myocardial damage.

Knowledge of the features of arrhythmias and typical changes in the ECG in the future can help physicians in the assessment and treatment of patients with COVID-19. It should be noted that changes in the electrocardiogram to predict heart damage in COVID-19 require further study.

Conclusions

1. It was found that the most common comorbid pathologies in patients with severe COVID-19 were - hypertension (67.7%), coronary heart disease (68.4%), chronic heart failure (48.9%), obesity (35.3%) and diabetes mellitus (4.1%).

2. The study found that the prevalence of cardiac arrhythmias in patients with severe COVID-19 was 67.7%.

3. Among all arrhythmias, tachyarrhythmias predominated - 51.1%, in the structure of which sinus tachycardia (48%) and atrial fibrillation (14.3%) were most common. Significantly more often recorded sinus tachycardia among women.

4. The prevalence of bradyarrhythmias was 45.1%, the structure of which was dominated by sinus bradycardia (12%) and RBBB (22.6%). Among men, there was a tendency to the predominance of LBBB.

5. The prevalence of ischemic changes and/or repolarization disorders according to ECG data was 89.5%, the structure of which was dominated by the presence of inversion of the T wave (68.4%) and depression of the ST segment (40.6%). In turn, the mean age of patients with ischemic ECG changes was significantly higher compared to patients without signs of repolarization disorders. Among men, the predominance of right ventricular hypertrophy and the tendency to predominate left ventricular hypertrophy were observed.

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ГЕНДЕРНО-ВІКОВІ ОСОБЛИВОСТІ ПОШИРЕНOSTI ТА СТРУКТУРИ ПОРУШЕНЬ СЕРЦЕВОГО РИТМУ У ПАЦІЄНТІВ З ТЯЖКИМ ПЕРЕБІГОМ COVID-ІНФЕКЦІЇ

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З'являється все більше доказів того, що аритмії є одним з основних ускладнень COVID-19 інфекції. Запалення міокарда, цитокіновий шторм, гіпоксія, зміни рівня електролітів, спазм коронарних судин, мікротромби можуть бути факторами, що сприяють появі порушень ритму та виникнення змін на електрокардіограмі. Метою роботи було встановити гендерно-вікові особливості поширеності та структури порушень серцевого ритму у пацієнтів з важким перебігом COVID-інфекції. У ході дослідження обстежено 133 пацієнтів з важким перебігом COVID-19 інфекції, які перебували у відділенні інтенсивної терапії міської клінічної лікарні №1 м. Вінниця у період з квітень - по грудень 2020 р. та у результаті лікування з покращенням самопочуття були виписані з лікарні. Статистичну обробку отриманих даних виконали за допомогою пакету статистичних програм SPSS 12.0 для Windows з використанням параметричних та непараметричних методів статистичного аналізу. Встановлено, що найчастішими коморбідними патологіями у пацієнтів з важким перебігом COVID-19 були - гіпертонічна хвороба (ГХ) 90 (67,7%), ішемічна хвороба серця (ІХС) 91 (68,4%), хронічна серцева недостатність (ХСН) 65 (48,9%), ожиріння 47 (35,3%) та цукровий діабет (ЦД) 32 (24,1%). Виявлено, що поширеність порушень серцевого ритму у пацієнтів з важким перебігом COVID-19 складала 86 (64,7%). Серед усіх порушень ритму переважали тахіаритмії - 68 (51,1%), у структурі яких найчастіше зустрічалася синусова тахікардія 45 (33,8%) та фібриляція передсердь - 19 (14,3%) пацієнтів, відповідно. У свою чергу, синусову тахікардію достовірно частіше реєстрували серед жінок. Поширеність брадіаритмії становила 60 (45,1%) випадків, у структурі яких переважали синусова брадикардія 16 (12%) та блокада правої ніжки пучка Гіса 30 (22,6%). Серед чоловіків спостерігалася тенденція до переважання повної блокади лівої ніжки пучка Гіса. Поширеність ішемічних змін та/або порушень реполяризації за даними ЕКГ становила 119 (89,5%), у структурі яких переважала наявність інверсії зубця Т 91 (68,4%) та депресії сегменту ST у 54 (40,6%) випадках. У свою чергу, середній вік пацієнтів з ішемічними змінами на ЕКГ був достовірно вищим, порівняно з пацієнтами без ознак порушень реполяризації. Серед чоловіків спостерігалася переважання гіпертрофії правого шлуночка та тенденція до переважання гіпертрофії лівого шлуночка.

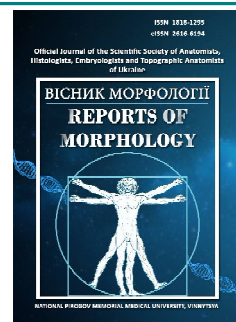
Ключові слова: COVID, порушення серцевого ритму, аритмія, фібриляція передсердь, екстрасистоля, блокади, гіпертонічна хвороба, ішемічна хвороба серця, хронічна серцева недостатність.



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Features of vaginal microbiocenosis in women of reproductive age with overweight and obesity

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*Overweight and obesity play a negative role in gynecological and obstetric practice. In women, the frequency of infectious pathology increases against the background of metabolic disorders. The most common form of infectious vaginitis is bacterial urogenital candidiasis, in the etiological structure of which a significant role belongs to the fungi *Candida albicans*, as well as *Candida non-albicans*: *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*. Associations of *Candida* fungi with various representatives of opportunistic microflora, such as gram-positive and gram-negative aerobic, facultative-anaerobic and obligate-anaerobic microorganisms, are often formed. As a result, numerous bacterial pathogens multiply and the number of lactobacilli, which are usually part of the bacterial flora of the vagina, is significantly reduced. In bacterial vaginosis (BV), the concentration of anaerobic pathogens *Peptostreptococcus* sp, *Gardnerella vaginalis*, *Peptostreptococcus Mobiluncus* sp, *Mycoplasma hominis* can increase 100 times. Activation of *Atopobium* vaginae and *Gardnerella vaginalis*, which play a "key" role in the pathogenesis of BV, has been proven. The aim of the study was to study changes in the vaginal microbiome in women with candidiasis and bacterial vaginosis in order to improve existing treatment regimens. We examined 120 women of reproductive age with overweight and obesity. The degree of microbial contamination was determined and the maximum possible spectrum of aerobic and facultative-anaerobic microflora was detected. In women with vulvovaginal candidiasis, overweight and obesity, a high concentration (lg5.8 CFU/ml) of *Candida* fungi was found, and in 95% of patients two-, three- and four-component associations of *Candida* fungi with various representatives of conditional pathogenic microflora. Lactobacillus deficiency was found in 58.3% of patients, and their complete absence - in 10.0%. Bacteriological examination of the vaginal contents of women with vaginosis and obesity revealed significant dysbiotic disorders of the vaginal microflora, three-, four- and even five-component associations of anaerobic and facultative anaerobic microflora with a predominance of anaerobes. A low seeding level of lactobacilli (lg2.2 CFU/ml) was established. Thus, the gram-positive aerobic and facultative anaerobic microflora of Firmicutes have a significant share in the spectrum of vaginal microflora in overweight and obese patients, in contrast to non-obese women of reproductive age. In women of reproductive age with vulvovaginal candidiasis and obesity, in contrast to non-obese patients, a higher frequency of fungal-bacterial associations, a higher quantitative level of vaginal contamination by *Candida albicans* and non-albicans with a lack or general absence of lactoflora.*

Keywords: overweight, obesity, microbiocenosis, bacterial vaginosis, urogenital candidiasis.

Introduction

Obesity is a significant medical and social problem of our time, the relevance of which is primarily related to the growing prevalence and the threat of the formation of various

pathological human conditions [1, 2, 18, 23, 27]. Obesity and overweight play an important negative role in gynecological and obstetric practice. There is information

that in women against the background of metabolic disorders increases the frequency of infectious pathology of various habitats, including the urogenital tract [6, 12, 19, 20, 28].

According to the prevalence of urogenital infections in women, one of the leading places is urogenital candidiasis [3, 21, 22, 26]. The most common causative agent of candidiasis is the fungus *Candida albicans*. But in the last decade in the etiological structure of candidiasis there is a tendency to increase the proportion of fungi *Candida non-albicans*: *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* [10]. A sign of modern genital candidiasis is the formation of associations of fungi of the *Candida* with various representatives of opportunistic pathogens. Associations with *Candida* fungi may include gram-positive and gram-negative aerobic, facultative-anaerobic and obligate-anaerobic microorganisms [29].

Firmicutes have a significant proportion in the bacterial spectrum of the vagina. Catalase-positive and coagulase-negative staphylococci in the vaginal microbiocenosis occur in more than 50% of women, *Streptococcus* spp. and *Enterococcus* spp. - in 30-40% of cases. Anaerobic gram-positive cocci (*Peptococcus* spp., *Peptostreptococcus* spp.) are detected with a frequency of 30-80%. Gram-negative anaerobic rod-shaped bacteria contaminate the female genital tract with considerable frequency: *Prevotella* spp., *Bacteroides* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Veillonella* spp [17]. Among enterobacteria, as factors of infectious pathology of genitals, a significant role belongs to *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and others [13]. Lactobacilli occupy a dominant position in the microflora of the vagina and cervix. *Bifidobacterium* are excreted from the genital tract with less frequency. Lactobacilli are a marker of the normal microbiocenosis of the genital tract. It is lactobacilli that determine the degree of colonization resistance of the vagina [8, 9, 15, 16].

One of the most common dysbiotic disorders of the vaginal microbiota is bacterial vaginosis (BV), which should be considered as an infectious non-inflammatory syndrome associated with dysbiosis of the vaginal habitat [5, 24, 25, 30].

The main manifestations of this pathology are a decrease or absence in the microflora of the vagina lactobacilli and activation of *Atopobium vaginae* and *Gardnerella vaginalis*, which play a "key" role in the pathogenesis of bacterial vaginosis. *Atopobium vaginae* is able to block factors of nonspecific reactivity (complement system), leads to increased inflammation in bacterial vaginosis, is a competitor of lactobacilli in terms of glycogen consumption. Data from the scientific literature indicate that metabolic disorders in women contribute to the active growth of *Gardnerella vaginalis*, which produce a specific toxin (cytolysin) that can cause desquamation of vaginal endothelial cells [4, 7, 14].

An important component of the bacterial spectrum in bacterial vaginosis is *Mobiluncus* spp. This microorganism

produces a toxin that inhibits the adhesion of lactobacilli on vaginal epithelial cells [11].

Given the high prevalence at the present stage of candidiasis and bacterial vaginosis of the genital tract, the negative impact of these diseases on the female reproductive system, the aim of our work was to study changes in vaginal microbiome in patients of these categories to improve existing treatment regimens.

Materials and methods

In order to solve the tasks of our work, we surveyed 120 women of reproductive age with overweight and obesity. For comparison, 60 women without overweight and obesity were examined. All women were divided into groups: Group I - women with vulvovaginal candidiasis and overweight and obesity (60 women); Group II - women with vulvovaginal candidiasis without excess weight and obesity (30 women); Group III - women with bacterial vaginosis and overweight and obesity (60 women); Group IV - women with bacterial vaginosis without excess weight and obesity (30 women). Data obtained from a survey of 30 healthy women were used as controls.

Carrying out of microbiological researches and the account of results was carried out according to the Order №234 of the Ministry of Health of Ukraine from 10.05.2007 and other regulatory documents.

The following differential diagnostic media were used to inoculate the genital mucus: blood agar, yellow-salt agar, Endo, Sabouraud agar, MRS medium for lactobacilli, Blaurok medium for bifidobacteria. Crops were carried out by the method of sector seeding on dense nutrient media, which allows to determine the degree of microbial contamination and to identify the maximum possible spectrum of aerobic and facultative anaerobic microflora.

Gardnerellosis was diagnosed by bacterioscopy by staining with Romanovsky smears with subsequent counting of "key" cells, amine test, determination of pH.

The contents of the vagina of women for the presence of fungi *Candida* were examined by microscopy and culture bacteriological method. Sabouraud agar was used to identify yeast-like fungi. MICROLA-TEST kits "Candidatest 21" (Erba Lachema s.r.o. (Czech Republic)) were used for further identification of isolated yeast-like fungi.

Determination of anaerobic microorganisms was carried out according to the guidelines "Laboratory diagnosis of purulent-inflammatory diseases caused by asporogenic anaerobic microorganisms", Kharkiv, 1985.

The anaerobic microflora of the genital tract was studied with strict adherence to the technique of anaerobic cultivation. Thioglycollate medium, blood agar with glucose, liver broth, Kitt-Tarozzi medium were used for the study. The "Anaerocult" system (Merck, Germany) and the "Anaerogas" gas packages were used to create anaerobic conditions. MICROLA-TEST "Anaerostest 23" kits (Erba Lachema s.r.o. (Czech Republic)) were used for further identification of isolated anaerobic microorganisms.

Identification of microorganisms was performed by their cultural and morphological characteristics. When identifying the isolated microorganisms, the Bergey's classification was followed.

To assess the contamination of the genital tract was carried out quantitative accounting of colonies that grew in dense nutrient media. The content of opportunistic pathogens in the test material was expressed by the number of colony-forming units in 1 ml (CFU/ml) of biological material.

Statistical analysis of the obtained research results was performed using standard computer packages "Data Analysis" Microsoft Excel for Windows 2002.

Results

The results of microscopy of the contents of the vagina in most women of group I with vulvovaginal candidiasis indicate negative changes in the microbiota, which have manifestations of vaginitis. In 83.3% of patients there was a significant increase in the number of neutrophils and leukocytes (30-100 in the field of view), increased epithelial desquamation, degenerative changes of the nuclei, vacuolation of the cytoplasm, deficiency or absence of normal morphotype microflora, a significant increase in gram-positive and gram-negative organisms. In 46.7% of the examined fungi were in the form of yeast cells, in 53.3% yeast and pseudomycelium were found.

In 6.7% of obese women, against the background of a moderate number of yeast cells, no manifestations of vaginitis were detected, but there was an accumulation of gram-positive cocci and gram-negative rods, as well as an increase in root-like bacteria and fusobacteria.

Examination of the contents of the vagina by bacteriological culture revealed that in patients of group I, *Candida* fungi were sown in all subjects at a significant concentration ($10^{5.8}$ CFU/ml).

In 95% of patients the formation of associations of fungi of the *Candida* with various representatives of opportunistic pathogenic microflora was revealed. Two-component bacterial-fungal associations were found in 28.3% of cases, three-component - in 48.3%, four-component - in 18.3% of patients. Monocultures of *Candida* fungi were registered in 5.1% of women.

Associative forms of vaginal dysbiosis differ from monomicrobial ones by greater aggressiveness, significant resistance to antibacterial drugs, severe structural changes in the vaginal mucosa.

As a result of bacteriological examination of women of group I, an increase in the composition of bacterial-fungal associations of the vaginal contents of Firmicutes, in particular, cocci with pathogenic properties was found. Thus, the frequency of the presence of *S.aureus* in the associations in women of group I reached 18.3%, epidermal staphylococcus with hemolysis - 23.3%, enterococcus - 20.0%.

Also, the associations of women of group I with a

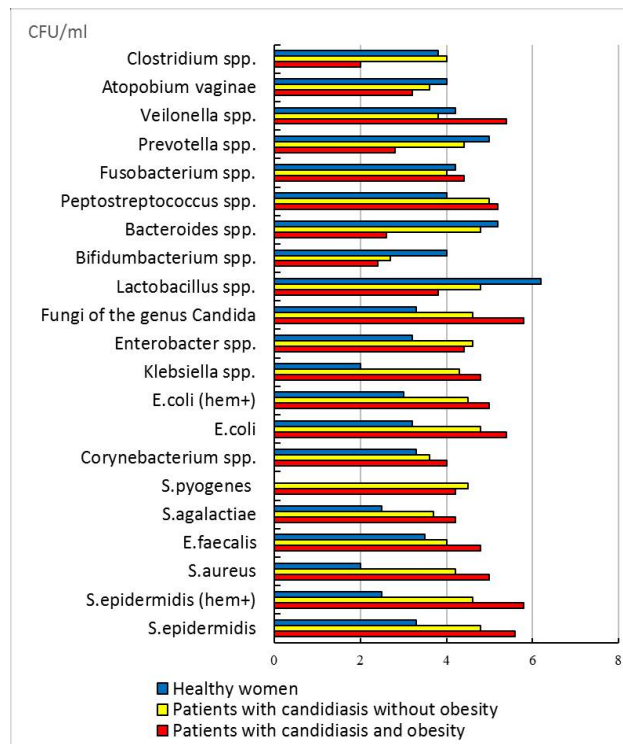


Fig. 1. Comparative diagrams of the composition of vagina microflora healthy women and women of reproductive age with vulvovaginal candidiasis and obesity.

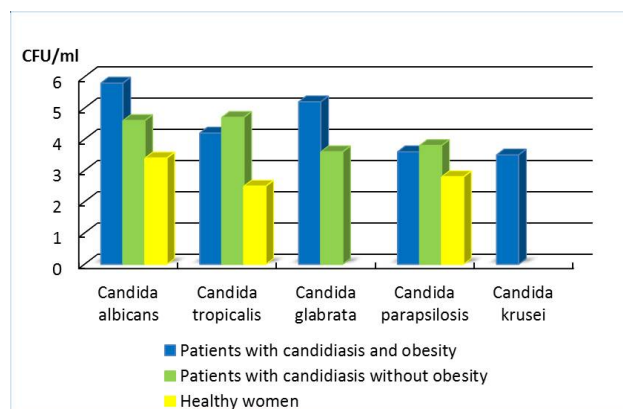


Fig. 2. Comparative diagrams of the species spectrum of *Candida* fungi of healthy women and women of reproductive age with vulvovaginal candidiasis and obesity.

significant frequency included different types of enterobacteria. A significant proportion was found in the associations of *Escherichia coli* with hemolytic properties (16.7%), *Klebsiella* spp. - 15%, *Enterobacter* spp. - 13.3%. Quantitative seeding rates of various representatives of opportunistic vaginal microflora in patients exceeded the diagnostic level ($10^{4.2}$ - $10^{5.8}$ CFU/ml) (Fig. 1).

Lactobacillus deficiency was found in 58.3% of patients, and their absence - in 10.0%. In 31.7% of cases, the registration rates of lactoflora were normal. Also in women of group I there was a decrease in the quantitative level of

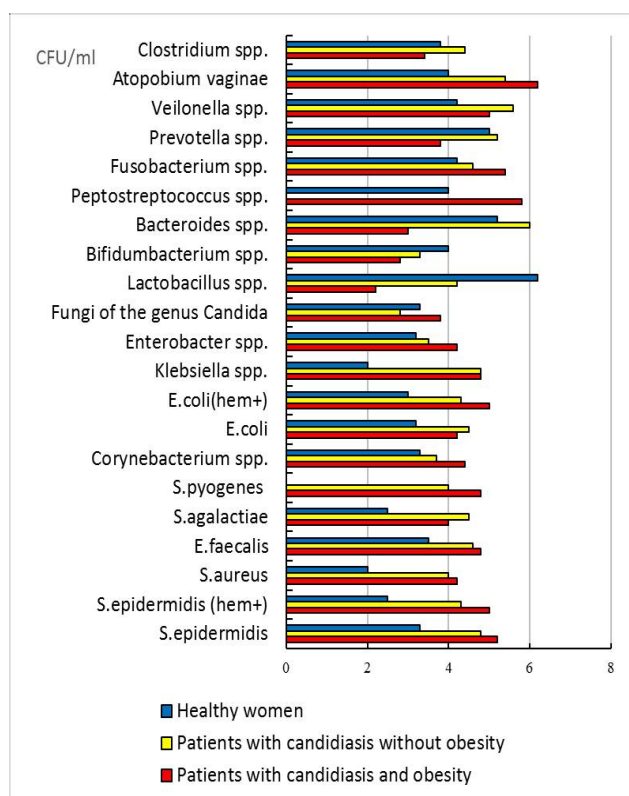


Fig. 3. Quantitative indicators of vaginal microflora of healthy women and women of reproductive age with bacterial vaginosis and obesity.

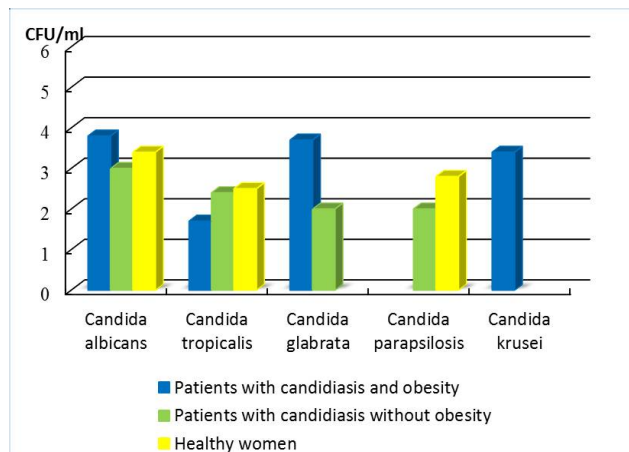


Fig. 4. Comparative diagrams of the species spectrum of *Candida* fungi of healthy women and women of reproductive age with bacterial vaginosis and obesity.

seeding lactobacilli.

According to the results obtained with the highest frequency, the vagina of patients of group I was contaminated with *Peptostreptococcus* spp. (28.3%), *Fusobacterium* spp. (16.7%) and *Veilonella* spp. (16.7%). The presence of *Bacteroides* spp. (13.3%), *Prevotella* spp. (10.0%), *Clostridium* spp. (6.7%) were registered with less frequency. The frequency of registration of *Gardnerella vaginalis* was only 3.3%, *Mobiluncus* spp. - 1.7%.

Quantitative seeding rates of individual members of the obligate anaerobic microflora exceeded the diagnostic level: *Peptostreptococcus* spp. - lg5.2 CFU/ml, *Veilonella* spp. - lg5.4 CFU/ml, *Fusobacterium* spp. - lg4.4 CFU/ml.

Quantitative level of vaginal contamination by *Bacteroides* spp., *Clostridium* spp., *Prevotella* spp. was reduced compared to normal (lg2.0 - lg2.8 CFU/ml).

In order to compare the indicators of vaginal microecology, the second group of patients with vulvovaginal candidiasis without obesity was examined.

Examination of the contents of the vagina in patients of group II by microscopy shows a less significant than in patients of group I, an increase in the number of leukocytes (15-50 in the field of view), moderate destructive changes in epithelial cells, lower frequency of pseudomycelial form of fungi (yeast cells - 76.7%, yeast cells and pseudomycelium - 33.3%). Microorganisms belonging to lactobacilli by morphotype were found more frequently in obese women than in group I patients.

According to the results of the study of vaginal contents by bacteriological method in women of the second group, despite the significant frequency of formation of fungal-bacterial associations (83.3%), the quantitative indicators of individual microbiocenosis in the subjects did not reach the level found in patients of group I. Two-component associations were found in 46.7% of the surveyed, three-component associations in 36.7% and monocultures in 16.6%.

The concentration of *Candida* fungi exceeded the norm and was lg4.6 CFU/ml. Gram-positive coccal microflora with pathogenic properties and enterobacteria were associated with fungi with a lower frequency than in patients of group I, and in low concentration. In women of group II, the frequency of vaginal contamination of *S.aureus* was 13.3%, *S.epidermidis* (hem+) - 16.7%, *Enterococcus faecalis* - 16.7%, *S.pyogenes* - 6.7%. The frequency of vaginal inoculation with enterobacteria in women was in the range of 10-16.7%. Quantitative indicators of Firmicutes with pathogenic properties exceeded the diagnostic level (>lg4.0 CFU/ml).

Some representatives of the anaerobic microflora in the vaginal associations in women of group II without obesity were registered with a higher frequency than in women of group I: *Bacteroides* spp. - 36.7%, *Clostridium* spp. - 10%, *Prevotella* spp. - 33.3%. The frequency of registration of *Gardnerella vaginalis* was 6.7%, *Mobiluncus* spp. - 3.3%, *Atopobium* spp. - 3.3%. Quantitative indicators of anaerobic seeding were in the range of lg3.6 - lg5.0 CFU/ml.

The rates of vaginal contamination with lactobacilli in women of group II were higher compared to women of group I. *Lactobacillus* deficiency was found in 46.7% of patients. Lactoflora was not determined in 6.7% of patients.

Examination of women in group I with vulvovaginal candidiasis and obesity revealed contamination of the vagina with various species of *Candida* non-albicans. *Candida glabrata* (10%) and *Candida tropicalis*

(6.7%) were detected with the highest frequency, and their quantitative indicators exceeded the diagnostic level. *Candida parapsilosis* and *Candida krusei* were shown with insignificant frequency (5% and 3.3%, respectively) (Fig. 2).

The species composition of *Candida non-albicans* in patients of the second group did not differ much from the species identified in women of group I. However, in the examination of women with vulvovaginal candidiasis without obesity, a lower frequency of vaginal inoculation with *Candida non-albicans* was found than in patients of group I. *Candida tropicalis* fungi were detected with the highest frequency (10%) and at a lg concentration of 4.7 CFU/ml in patients of group II. Other species of non-albicans in women of group II were registered with less frequency and in low concentration. *Candida krusei* was not detected in the spectrum of non-albicans fungi isolated from the vagina in patients of group II.

The task also included the study of the peculiarities of changes in the microbiocenosis of the vagina in patients with bacterial vaginosis and obesity (group III). As a comparison, patients with vaginosis without obesity (group IV) were examined.

The results of microscopy of the contents of the vagina in women of group III with vaginosis indicate a significant presence of parabasal epithelial cells and "key" cells, the phenomenon of desquamation of the squamous epithelium, a significant density of coccal microflora and rod-shaped leukocytes in the vaginal material were registered in moderation (15-35 in the field of view). Morphotypes of microflora corresponding to lactobacilli were detected with insignificant frequency or were absent. The frequency of registration of fungi by microscopy did not reach a high level (yeast cells - 15%, yeast + pseudomycelium - 8.3%).

Bacteriological examination of the contents of the vagina in patients of group III with vaginosis and obesity allowed to establish significant dysbiotic disorders of the vaginal microflora.

The results of the study of vaginal contents in patients of group III show that overweight and obesity are associated with a significant decrease in the frequency and quantitative indicators in the spectrum of vaginal microbiota of Bacteroidetes phylum, which includes Bacteroides and Prevotellaceae family and increased vaginal microflora representatives of Firmicutes.

Associations of anaerobic and facultative-anaerobic microflora with dominance of anaerobes were revealed in all examined patients of the III group. Three-component associations of bacterial flora were observed in 38.3% of patients, four-component associations in 45%, and five-component associations in 16.7%.

Examination of the contents of the vagina showed that with the highest frequency in patients of group III were found *Gardnerella vaginalis* (83.3%), *Atopobium vaginae* (68.3%), *Mobiluncus* spp. (41.7%). The presence of *Atopobium* in the spectrum of vaginal microflora is a sign of recurrent disease. Also in the composition of bacterial associations in patients of group III, an increase in the frequency of

registration of *Peptostreptococcus* spp. (43.3%), *Fusobacterium* spp. (36.7%), *Veillonella* spp. (31.7%). *Bacteroides* spp., *Prevotella* spp. and *Clostridium* spp. were determined in associations with insignificant frequency (15.0%, 11.7% and 8.3%, respectively).

Quantitative indicators of vaginal contamination by obligate anaerobes in patients of group III significantly exceeded the norm. The diagnostic level of *Peptostreptococcus* spp., *Fusobacterium* spp., *Veillonella* spp., *Atopobium vaginae* exceeded the diagnostic level. Whereas quantitative seeding rates of *Bacteroides* spp. were at a low level - lg3.0 CFU/ml (Fig. 3).

Among gram-negative facultative anaerobic rods in the associations of women with vaginosis with the highest frequency were *E. coli* (18.3%), *E. coli* hem+ (13.3%), *Klebsiella* spp. (16.7%). Quantitative indicators of Firmicutes group III patients and intestinal microflora isolated from the vagina exceeded the diagnostic level (lg4.0 - lg5.0 CFU/ml).

In 16.7% of the surveyed women of group III, the presence of *Candida* fungi was detected in insignificant concentration (lg3.8 CFU/ml). The spectrum of *Candida* fungi included *C. albicans* - 8.3%, *C. glabrata* - 5.0%, *C. tropicalis* - 1.7%, *C. krusei* - 1.7% (Fig. 4).

Dysbiotic changes of the vaginal microbiota in patients of group III were characterized by a significant decrease in vaginal contamination by representatives of the normal microflora - lactobacilli. The seeding rate of lactobacilli was 56.7%, absence - 43.3%. Quantitative seeding rates of lactobacilli were low (lg2.2 CFU/ml).

For comparison, we examined patients with vaginosis without obesity (group IV). According to microscopy, no significant leukocyte reaction was detected in the vaginal material (5-25 in sight). There was a significant increase in the number of "key" cells and an increase in the density of vaginal contamination with gram-positive and gram-negative microflora.

Bacteriological examination of the vaginal microbiome in patients with obesity without vaginosis revealed a lower frequency and lower quantitative level of vaginal contamination with obligate anaerobes and facultative anaerobic microflora. The anaerobic spectrum of the vaginal microflora included *Gardnerella vaginalis* (73.3%), *Atopobium vaginae* (43.3%), *Mobiluncus* spp. (30.0%), *Peptostreptococcus* spp. (33.3%) and *Fusobacterium* spp. (20.0%). The frequency of registration of anaerobes exceeded that found in patients of group III: *Bacteroides* spp. - 56.7%, *Veillonella* spp. - 46.7%, *Clostridium* spp. - 46.7%.

Quantitative indicators of obligate anaerobic microflora isolated from the vagina of women of group IV were in the range of lg4.4 - lg6.0 CFU/ml. But in women of group IV seeding rates of *Bacteroides* spp. and *Prevotella* spp. significantly exceeded those found in patients of group III (respectively lg6.0 - lg5.2 CFU/ml).

With a lower frequency than in patients of group III, in patients of group IV, the microbial associations included individual representatives of the intestinal microflora: *E. coli*

- 13.3%, *E.coli* hem+ - 10.0%, *Klebsiella* spp. - 13.3%, *Enterobacter* spp. - 6.7%.

The frequency of seeding from the vagina of Firmicutes patients, in particular, gram-positive coccal microflora did not reach significant values: *S.aureus* - 10.0%, *S.epidermidis* (hem+) - 13.3%. Quantitative indicators of vaginal seeding of facultative-anaerobic microflora were in the range of lg3.5 - lg4.8 CFU/ml.

The contents of the vagina in women of group IV with a low frequency (16.7%) and a small amount included *Candida* fungi (lg2.8 CFU/ml). *Candida albicans* was detected with the highest frequency (6.7%). The frequency of registration of fungi *Candida non-albicans* was: *C.tropicalis* - 3.3%, *C.parapsilosis* - 3.3% (Fig. 4).

In patients of group IV, the indicators of vaginal contamination with lactobacilli did not reach the norm, but were at a higher level (76.7%) than in patients of group III in the amount of lg4.2 CFU/ml.

The composition of associations in patients of group IV was represented by 2-4 types of microflora: two-component associations (16.7%), three-component (50%), four-component (33.3%). Five-component bacterial associations were not determined.

Discussion

Clinical assessment of the condition of the lower genitalia without taking into account the data of microscopy is subjective and does not always allow to detect vaginal dysbiosis in patients [29].

The results of examination of women with vaginosis (groups III and IV) show that obese patients form multicomponent anaerobic-aerobic bacterial communities with a significant content of *Gardnerella vaginalis*, *Atopobium* vaginae, as well as Firmicutes with pathogenic properties on the background of low or no normal microflora. As S.I. Klimnyuk and co-authors pointed out in their study (2019): not only *Gardnerella vaginalis* but also anaerobes *Mobiluncus* spp., *Bacteroides* spp., *Mycoplasma hominis* and other microorganisms are involved in the development of vaginal dysbiosis, and the only cause of bacterial vaginosis there is a violation of the quantitative composition of microorganisms in the vaginal environment on the background of other dysbiotic processes in the body. Complications associated with bacterial vaginosis include chronic inflammation of the internal genitals, the development of adhesions of the pelvic organs, infertility [17].

Some studies suggest that a higher body mass index is associated with increased susceptibility to bacterial vaginosis, but the results are conflicting. Prior to his open cohort study conducted in Mombasa, Kenya, Lookken E.M. with co-authors in 2019 [19] selected women sex workers aged 16 to 45 years. Women who were obese, the authors concluded, had an almost 20% lower risk of developing bacterial vaginosis compared to women with a normal body mass index. The authors continue to suggest that it is appropriate to study the potential mechanisms of this effect,

including the possible effects of diet related to obesity changes in the intestinal microbiome and systemic estrogen levels [19].

At the same time, R.T.Brookheart and co-authors in 2019 [6] showed that bacterial vaginosis is one of the most common vaginal diseases in the United State. Recent studies have shown that in obese women, the abnormal microbiota resembles bacterial vaginosis; however, few studies have examined the prevalence of bacterial vaginosis in overweight and obese population. Moreover, it is not known whether there are racial differences in the ratio of obesity to bacterial vaginosis. In their study, the authors examined the correlation between body mass index and bacterial vaginosis, as determined by the Nugent scale, and identified the influence of race. Overweight and obese women were found to have a higher incidence of bacterial vaginosis than lean women, even after adjusting for race-related variables. Among white women, the prevalence of bacterial vaginosis was higher among overweight women and white women with class I and II/III obesity compared to lean white women, a phenomenon not observed among black women. The authors concluded that overweight and obese women had higher Nugent scores and higher levels of bacterial vaginosis compared to lean women. Interesting was the fact that black women had a higher prevalence of bacterial vaginosis, regardless of their body mass index, compared to white women [6].

Obesity is associated with an increased level of susceptibility to infections, however, the results of clinical studies assessing body mass index and vaginitis are contradictory, G.Ventolini and co-authors pointed out in 2017. They found an association between obesity and recurrent vulvovaginal bacterial infections in women of childbearing potential. The authors proved that obesity can be an independent risk factor for vulvovaginal bacterial infections in women of reproductive age due to altered vaginal immunity [28].

In general, the results of the study of vaginal microbiota in obese women allowed us to identify a significant frequency of two variants of microbiocenosis: candidal vaginitis and vaginal vaginosis, which is confirmed by other studies [21, 27]. The identified variants of vaginal microbiome disorders in patients of our study groups have differences in species and quantitative composition of microbial associations, different indicators of vaginal contamination by *Candida* fungi, different degrees of imbalance between potentially pathogenic and normal microflora, as indicated by other researchers [24, 28].

The obtained data indicate the feasibility of further development of pathogenetically sound schemes for the treatment of disorders of the vaginal microbiocenosis in obese women.

Conclusions

1. The composition of vagina microbiota in women of reproductive age with overweight and obesity (groups I and

II) differs from the composition of the microbiota in women of reproductive age without obesity (groups III and IV) by statistically significant decrease in vaginal contamination by anaerobic microflora, which has a significant metabolic potential: *Bacteroides* spp. and *Prevotella* spp. Gram-positive anaerobic and facultative-anaerobic microflora of Firmicutes have a significant share in the spectrum of vaginal microflora in overweight and obese patients, in contrast to non-obese women of reproductive age.

2. In women of reproductive age with vulvovaginal candidiasis who are obese (group I), in contrast to non-obese patients (group II), a higher frequency of fungal-bacterial associations, a higher content in the associations of Firmicutes with pathogenic properties and

Proteobacterium, a higher quantitative level of vaginal contamination by the fungi *Candida albicans* and non-*albicans* on the background of deficiency or lack of lactoflora observed.

3. The state of microecology of the vagina in women of reproductive age with bacterial vaginosis, overweight and obesity (group III) is characterized by a significant frequency of multicomponent associations, a significant imbalance between the associates of obligate-anaerobic microflora. An increase in vaginosis-associated microflora (*Atopobium* vaginae, *Gardnerella vaginalis*, *Mobiluncus* spp.) and a significant deficiency of *Bacteroides* spp., *Prevotella* spp., *Lactobacillus* spp. was established.

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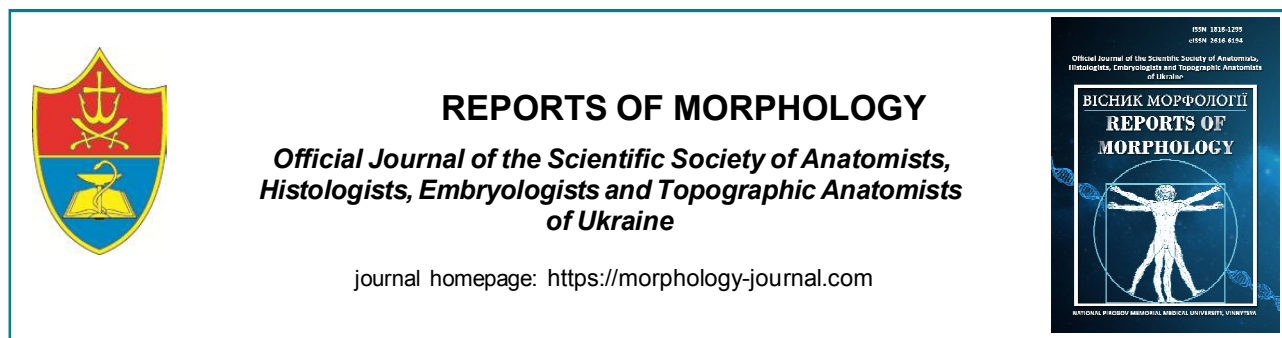
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ОСОБЛИВОСТІ МІКРОБІОЦЕНОЗУ ПІХВИ У ЖІНОК РЕПРОДУКТИВНОГО ВІКУ З НАДЛИШКОВОЮ МАСОЮ ТІЛА ТА ОЖИРІННЯМ

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Надлишкова вага та ожиріння відіграють неагативну роль в гінекологічній та акушерській практиці. У жінок збільшується частота інфекційної патології на фоні метаболічних розладів. Найбільш розповсюджена форма інфекційного вагініта - це бактеріальний урогенітальний кандидоз, в етіологічній структурі якого визначна роль належить грибам *Candida albicans*, а також *Candida non-albicans*: *C.glabrata*, *C.tropicalis*, *C.parapsilosis*, *C.krusei*. Нерідко формуються асоціації грибів *p.Candida* з різними представниками умовно патогенної мікрофлори, такими як грампозитивні та грамнегативні аеробні, факультативно-анаеробні та облигатно-анаеробні мікроорганізми. Внаслідок цього розмножуються багаточисленні бактеріальні збудники та суттєво зменшується кількість лактобактерій, які зазвичай є у складі бактеріальної флори вагіни. При бактеріальному вагінозі концентрація анаеробних патогенів *Peptostreptococcus sp*, *Gardnerella vaginalis*, *Peptostreptococcus Mobiluncus sp*, *Mycoplasma hominis* може збільшуватись у 100 разів. Доведено активацію *Atopobium vaginae* та *Gardnerella vaginalis*, що відіграють "ключову" роль у патогенезі БВ. Метою роботи стало вивчення змін мікробіому піхви у жінок з кандидозом та бактеріальним вагінозом з метою удосконалення існуючих схем терапії. Обстежили 120 жінок репродуктивного віку з надлишковою вагою та ожирінням. Визначали ступінь мікробного обміненія та виявляли максимально можливий спектр аеробної та факультативно-анаеробної мікрофлори. У жінок з вульвовагінальним кандидозом, надлишковою вагою і ожирінням встановлена висока концентрація (lg5,8 КУО/мл) грибів *p.Candida*, а у 95% хворих формувались двух-, трьох- та чотирьохкомпонентні асоціації грибів *p.Candida* з різними представниками умовно-патогенної мікрофлори. Дефіцит лактобацил встановлений у 58,3% хворих, а їх повна відсутність - у 10,0%. Бактеріологічне дослідження вмісту піхви жінок з вагінозом та ожирінням виявило значні дисбіотичні порушення складу піхвової мікрофлори, трьох-, чотирьох- та навіть п'ятикомпонентні асоціації анаеробної та факультативно-анаеробної мікрофлори з домінуванням анаеробів. Встановлений низький рівень висіву лактобацил (lg2,2 КУО/мл). Таким чином, значну питому вагу в спектрі мікрофлори піхви у пацієнток з надлишковою вагою та ожирінням, на відміну від жінок репродуктивного віку без ожиріння, має грампозитивна анаеробна та факультативно-анаеробна мікрофлора філуму *Firmicutes*. У жінок репродуктивного віку з кандидозним вульвовагінітом та ожирінням, на відміну від пацієнток без ожиріння, встановлена більш висока частота грибово-бактеріальних асоціацій, більш високий кількісний рівень контамінації піхви грибами *Candida albicans* та *non-albicans* на фоні дефіциту або загальної відсутності лактофлори.

Ключові слова: надлишкова маса тіла, ожиріння, мікробіоценоз, бактеріальний вагіноз, урогенітальний кандидоз.



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Gallbladder cholesterolosis in patients with metabolic syndrome and chronic pancreatitis

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Cholesterolosis, as a pathological condition caused by metabolic disorders, is potentially associated with metabolic syndrome and chronic pancreatitis. The aim of the study was to assess the prevalence and morphological features of gallbladder cholesterolosis in the contingent of patients with metabolic syndrome and chronic pancreatitis. 82 patients who underwent cholecystectomy for chronic calculous cholecystitis were included in our study. The experimental group consisted of 37 patients with metabolic syndrome and chronic pancreatitis. The comparison group included 45 patients without clinically confirmed metabolic syndrome and chronic pancreatitis. In accordance with the purpose and objectives, morphological examination of the gallbladder wall was performed. Data to be analyzed were processed using the statistical software package SPSS 20.0 for Windows. The gender and age structure of the studied contingent did not differ from that described in the scientific literature. The morphological picture of cholesterolosis did not differ in the study groups. In a mucous membrane considerable polymorphism of folds (villi) was noted. There were signs of desquamation and regeneration in the epithelium. There was a violation of the structure Rokitansky-Aschoff sinuses. In some crypts microconcretions were defined. Atrophy and sclerosis of the stroma of the villi were detected in the mucous membrane. Significant expansion of the lumen of the veins in the own plate of the mucous membrane and the perimuscular layer of the gallbladder wall was noteworthy. Signs of stasis were found in the lumen of blood vessels. The lumen of the arteries was narrowed in places due to swelling and accumulation of yellow-brown pigment. Signs of spasm and significant sclerosis of the wall were identified. Dystrophic changes in the endothelium contribute not only to the development of uneven plethora of stagnant nature with significant edema, but also hemorrhage. Significant swelling of all layers was characteristic, causing their disintegration. Significant dilation of lymphatic vessels causes a significant thickening of the gallbladder wall. In some places, there is uneven hypertrophy of the muscle layer. There was an excessive increase in the tortuosity ducts of Luschka. According to the study, patients with metabolic syndrome and chronic pancreatitis, operated on for chronic calculous cholecystitis, along with maintaining the gender and age structure of the contingent, there is a significantly higher incidence of gallbladder cholesterolosis.
Keywords: gallbladder cholesterolosis, metabolic syndrome, chronic pancreatitis, morphology.

Introduction

Despite the fact that gallbladder cholesterolosis was described in 1857 by R. Wirhov, for a long time this pathology was little known to practitioners and was considered a rare and difficult to diagnose disease. For almost a century and a half, all scientific publications covering gallbladder cholesterolosis were mostly descriptive, and the pathology itself was only accidental findings in pathomorphological examination.

Interest in this problem has increased especially in the last two decades, when research began to appear aimed at clarifying the etiopathogenetic mechanisms of this pathology. This served as an impetus for the development of diagnostic criteria, tactics of such patients and methods of treatment [29].

The frequency of detection of this pathology, according to various authors, varies in an extremely wide range - from

3-5% to 50% or more [7, 18]. This fact can be explained both by the difficulty and specificity of diagnostic measures, and the lack of clear protocols for early detection of cholesterolosis [31].

Cholesterolosis can be localized in the form of a "cholesterol polyp", or have a diffuse spread in the mucous membrane [16]. In addition, the changes inherent in cholesterolosis include signs of many local pathological processes. Thus, in the literature there are no clear differential differences between "cholesterol polyp" and sclerosed and deformed villi (folds) [26].

The development of cholesterolosis is associated with disorders of lipid metabolism [1, 25]. At the same time, such disorders accompany the development of metabolic syndrome [11, 14, 17, 19, 22, 23], the relationship of which with cholesterolosis is currently well studied [8, 12].

Without creating a direct significant negative impact on the general condition of the body, gallbladder cholesterolosis is a risk factor for a number of serious diseases. Thus, the role of this pathology in the development of acute pancreatitis has been proven [3, 20]. Acute pancreatitis, in turn, leads to a significant increase in the risk of developing chronic pancreatitis and pancreatic cancer [10, 13, 15, 28, 30]. There are some conflicting views in the literature regarding the association of cholesterolosis with gallbladder cancer. Some studies show a direct link between the frequency of cholesterolosis findings and the development of a neoplastic process in the gallbladder [4], other studies indicate an extremely low incidence of gallbladder cancer in this group of patients [21].

The incompletely studied pathogenetic and clinical-laboratory connections of gallbladder cholesterolosis with metabolic pathology and diseases of the gastrointestinal tract encourage research in this area. In our opinion, the study of gallbladder cholesterolosis in patients with metabolic syndrome and chronic pancreatitis is relevant.

The aim of the study was to assess the prevalence and morphological features of gallbladder cholesterolosis in the contingent of patients with metabolic syndrome and chronic pancreatitis.

Materials and methods

Prospective clinical research was performed at the Department of Endoscopic and Cardiovascular Surgery of National Pirogov Memorial Medical University, Vinnytsya on the basis of the Clinical Highly Specialized Surgical Center with Minimally Invasive Technologies of Vinnytsya Regional Clinical Hospital named after M.I. Pyrogov.

According to the purpose and objectives, the study included 82 patients who underwent cholecystectomy for chronic calculous cholecystitis. The mean age of patients was 57.0 ± 13.2 years. There were 58 women (70.73%) and 24 men (29.27%) in the studied contingent.

The experimental group consisted of 37 patients with metabolic syndrome and chronic pancreatitis. The comparison group included 45 patients without clinically

confirmed metabolic syndrome and chronic pancreatitis.

The gallbladder, removed by cholecystectomy, was dissected lengthwise after surgery, the contents removed, and flushed with running water. Macroscopic changes in the shape, size, condition of the mucous membrane were described in the postoperative protocol. Signs of cholesterolosis were considered to be the presence of a yellow mesh in the mucous membrane on a red background. The prevalence of the described changes more than 2 centimeters was diagnosed as diffuse cholesterolosis, at smaller sizes - as focal. Attention was drawn to the presence of polyps. Given that the mucosa is normally velvety, not smooth, the assessment of polyps on examination of the mucosa is subjective, as polyps may be small, smaller than normal villi, so polyposis cholesterolosis is best diagnosed by histological examination.

After macroscopic examination, the gallbladder was fixed in a 10% solution of neutral formalin. After fixation for 3 days, gallbladder preparations were prepared according to standard methods. Paraffin sections 5-7 μ m thick were stained with hematoxylin and eosin. Microscopy and photographing of histological specimens were performed using a light microscope OLIMPUS BX 41 at magnifications of 40, 100, 200, 400 and 1000 times. Image acquisition and processing, as well as morphometry were performed using the program "Quick PHOTO MICRO 2.3".

Data that were subject to statistical analysis were processed using the statistical software package SPSS 20.0 for Windows.

Results

The mean age of patients in the experimental group was 56.8 ± 12.5 years. There were 26 women in the group (70.27%), 11 men (29.73%). The mean age of patients in the comparison group was 57.1 ± 13.9 years. There were 32 women (71.11%) in the group, 13 men (28.89%).

Regarding the prevalence of cholesterolosis in the studied group of patients, in the experimental group of such patients there were 15 (40.54%), in the comparison group - 8 (17.8%). The differences between the groups were statistically significant ($p < 0.05$).

When analyzing the sex distribution of patients with cholesterolosis, it was found that in the experimental group there were 9 (60%) women and 6 (40%) men, and in the comparison group - 6 (75%) women and 2 (25%) men. Statistically significant differences were not proved ($p > 0.05$).

Regarding the dependence of the incidence of gallbladder cholesterolosis on the age of patients, we did not find any statistically significant patterns.

In the morphological study in both the experimental group and the comparison group, the following changes were observed.

In patients operated on for chronic calculous cholecystitis by examination microscopy of the gallbladder wall (bottom of the body and neck) found quite similar

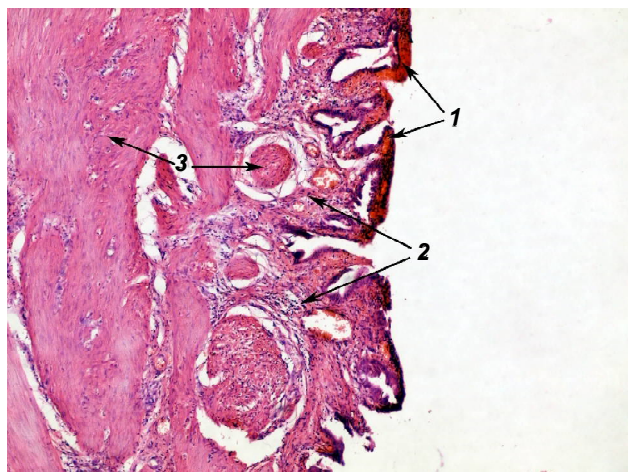


Fig. 1. Lack of a clear boundary between the mucous membrane (1) perimuscular (2) and the muscular structural elements (3) of the gallbladder wall. Hematoxylin-eosin. x100.

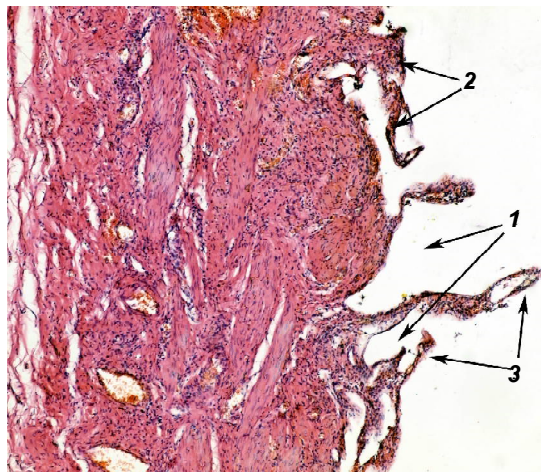


Fig. 2. Uneven narrowing and widening of the lumen of the crypt of the mucous membrane (1), sclerosis of the stroma of the villi (2), on the surface of which the epithelium is flattened or absent (3). Hematoxylin-eosin. x100.

morphological changes that differ only in depth, distribution and ratio. Thus, in the wall of the gallbladder of all areas there are significant violations of all layers, which is manifested by the disintegration of the classical ratio of membranes due to the unevenness of inflamed atrophic, hypertrophic, sclerotic and hemodynamic processes (Fig. 1).

The mucous membrane acquires especially significant changes in the conditions of chronic calculous cholecystitis. In almost all cases, signs of cholesterolosis of varying severity, both in the mucous membrane and in other layers of the gallbladder wall. In the mucous membrane there is a significant polymorphism of folds (villi), which are presented in the form of both high and narrow, and wide and low structures. These structures are often deformed and lined, mainly with flattened cubic, less often prismatic, epithelium, under which significant sclerosis is often defined (Fig. 2).

There are signs of desquamation and regeneration in the epithelium. The latter is manifested by a layering of

cellular elements with hyperchromia of the nuclei. Normally, the glandular structures of the mucous membrane (crypts) are placed vertically with a certain frequency, depending on the location (bottom, body, neck). They are lined with prismatic mucus-forming epithelium, which provides a mucus-forming function aimed at liquefying bile. In our observations, a significant violation of the structure of the crypts and Rokitansky-Aschoff sinuses draws attention. They are significantly deformed, often cystically enlarged, and the epithelium lining them is either flattened or absent at a great distance due to its desquamation (Fig. 3).

In some crypts on the surface of the epithelium or in the lumen are defined by dense orange mass of irregular shape (microconcretions) (Fig. 4).

In the cytoplasm of cells, the surface of the villi and crypts, where the epithelium is absent, as in epithelial cells, are determined by dusty or granular inclusions of brownish-

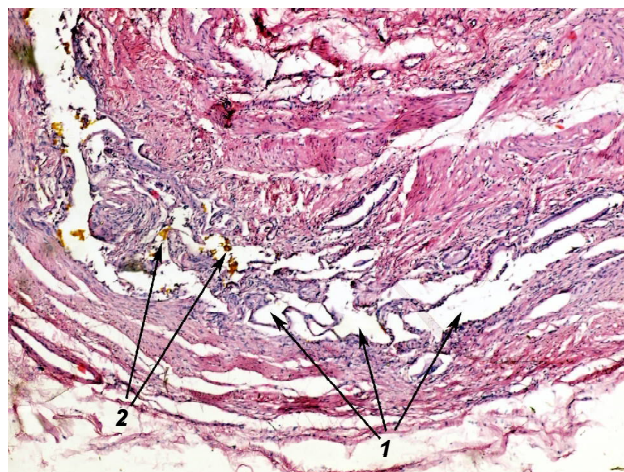


Fig. 3. Cystic enlargement and deformation of the Rokitansky-Aschoff sinuses (1), in desquamated epitheliocytes pigment inclusion (2). Hematoxylin-eosin. x100.

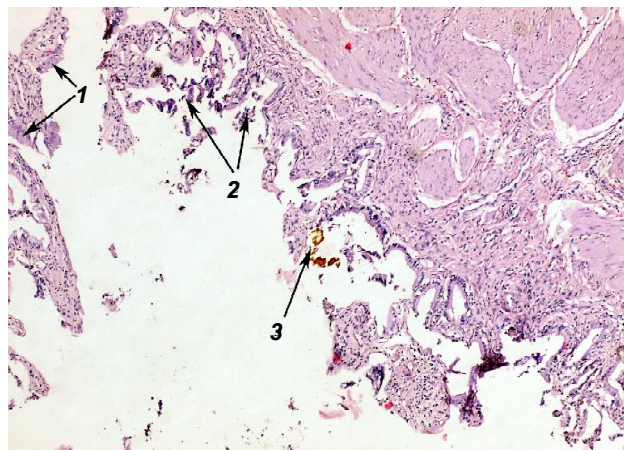


Fig. 4. Swelling (1) and desquamation (2) of the crypt mucosa epithelium of the gallbladder, fixed unstructured masses (microconcretions) of orange color (3) in place of the desquamated epithelium. Hematoxylin-eosin. x200.

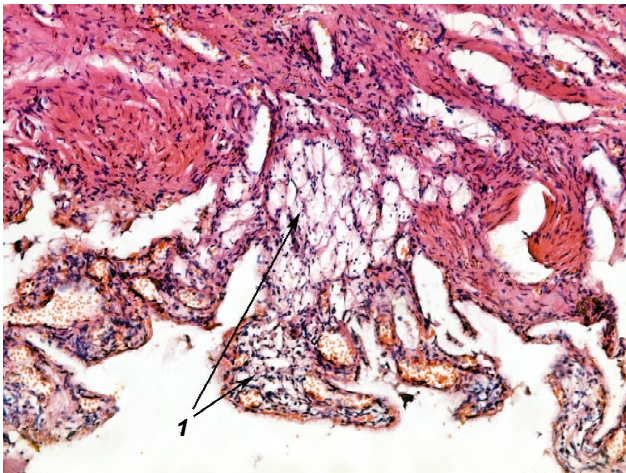


Fig. 5. Numerical xanthoma cells (1) in the stroma of villi. Hematoxylin-eosin. x200.

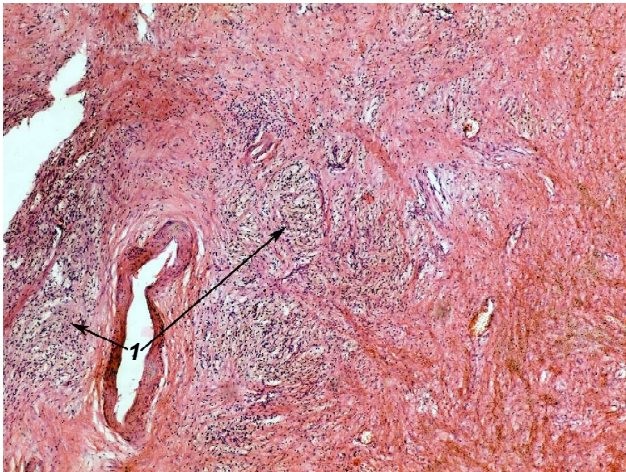


Fig. 6. Focal lympho-histiocytic infiltration (1), by type of xanthogranuloma. Hematoxylin-eosin. x200.

brown color. There are accumulations of desquamated epithelium among the condensed yellow bile, which also differs from the normal accumulation of pigment (xanthoma cells). In the mucous membrane is constantly detected atrophy and sclerosis of the stroma of the villi (folds), which is represented by fibroblasts and densely spaced collagen fibers, between which there are "foamy" cells and lymphocytes (Fig. 5). Accumulations of such cells in some patients are found in the adventitia and in the muscle layer.

Blood vessels in the stroma of the villi are sometimes not identified. As for the own plate of the mucous membrane, the number of blood vessels in the microcirculatory tract is uneven. In some cases, they are more than normal, in others - they are almost absent. Draws attention to the significant expansion of the lumen of the veins in its own plate of the mucous membrane and the perimuscular layer of the gallbladder wall. Signs of stasis and uneven distribution of cellular elements (erythrocytes) and plasma are found in the lumen of blood vessels of different calibers, which is a morphological reflection of gallbladder wall dystonia. As for

the arteries, which are known to be mainly localized in the perimuscular layer, their lumen is narrowed in places due to swelling and accumulation of yellow-brown pigment. There is a recalibration of the arteries in the direction of reducing the caliber, due to swelling and proliferation of endothelial cells. Signs of spasm and significant sclerosis of the wall are identified. In some vessels, endothelial cells in the presence of dystrophy significantly narrow the lumen and contribute to the development of stasis, and sometimes fragmentarily obliterate it over a fairly long period.

Dystrophic changes in the endothelium contribute not only to the development of uneven plethora of stagnant nature with significant edema, but also hemorrhage. Xanthoma cells are also found in arterioles, the obliteration of which is accompanied by wall necrosis, and sometimes causes the development of small foci of fibrinoid necrosis in the surrounding tissues, which is more common in gallbladder adventitia. Focal changes of an alternative nature initiate the development of inflammatory, focal or diffuse infiltration mainly by mononuclear cells: lymphocytes, histiocytes, macrophages. Structures resembling xanthoma granulomas are formed (Fig. 6).

Gallstone disease and cholesterolosis are characterized by significant swelling of all layers, which causes their disintegration. Significant dilation of lymphatic vessels causes a significant thickening of the gallbladder wall. Numerous lipid patches were sometimes observed in the lumen of the lymphatic vessels of the adventitia (Fig. 7).

In some places, there is uneven hypertrophy of the muscle layer. Smooth muscle fibers are unevenly stained with eosin. Their cytoplasm is often vacuolated.

In some cases, especially large lacunae of edematous fluid are found in the mucous membrane's own plate and in the muscular layer, which cause disintegration of tufts by drag, which have different thickness, different direction with predominance of muscle bundles in the transverse (circular) direction. Muscle fiber dystrophy is evidenced by the

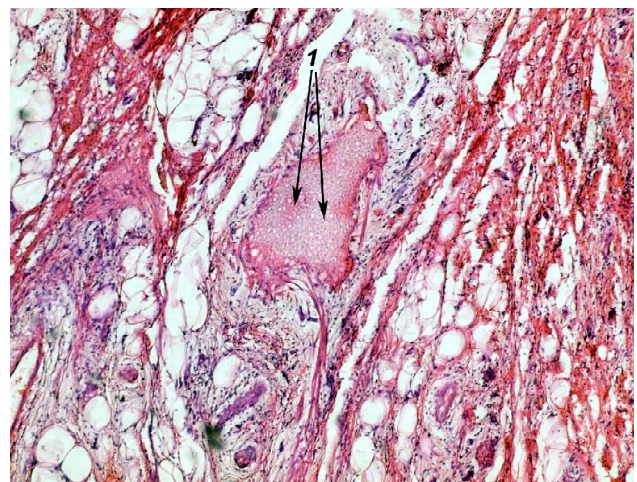


Fig. 7. Numerous lipid droplets (1) in the lumen of the dilated lymphatic vessel of the gallbladder adventitia. Hematoxylin-eosin. x100.



Fig. 8. Localized in the muscular layer dilated glands (1) lined with prismatic epithelium on the background of atrophy of the mucous membrane (2). Hematoxylin-eosin. x100.

heterogeneous intensity of sarcoplasmic staining and hypochromia of the nuclei. In other cases, the muscle layer is represented by densely spaced bundles of fibers that are unevenly bounded by fibrous tissue. Swelling in such cases is insignificant or absent. There are fields of fibrous tissue with signs of hyalinosis. Rarely, single tubular-type glands with a dense basement membrane, with moderate lumen enlargement and swelling of the epithelium or foci of pseudoadenomatosis are identified in the muscular layer, represented by clusters of tubular-type glands lined with cubic epithelium (Fig. 8). There is an excessive increase in the tortuosity of ducts of Luschka.

Discussion

The pathogenesis of gallbladder cholesterosis is characterized by the presence of a large number of links in pathological processes, and their relationship is not fully described in the modern scientific literature [31].

In our study, no age-specific features of the development of gallbladder cholesterosis were found, and the sex distribution showed results similar to those described in the scientific literature [7].

Instead, it was confirmed that patients with metabolic syndrome and chronic pancreatitis have a higher incidence of gallbladder cholesterosis. It is known that the metabolic syndrome is accompanied by a violation of all types of metabolism [1, 25]. Changes in metabolism, of course, are reflected in the qualitative and quantitative composition of bile [8, 12], which can, at least in part, contribute to the development of changes in the gallbladder.

As for the relationship between chronic pancreatitis and gallbladder cholesterosis, it is still difficult to unambiguously determine the root cause of the changes. The anatomical unity of the hepatopancreatobiliary system determines the simultaneous and parallel development of pathological processes in the liver, biliary tract and pancreas. Changes in one part of this system contribute to

physiological and anatomical disorders in the activities of others. That is why, on the one hand, fibrosis of the tissues of the pancreas can disrupt the outflow of bile from the gallbladder, followed by the creation of favorable conditions for the formation of stones. On the other hand, changes in the gallbladder wall lead to increased formation of stones in its lumen and are often the cause of episodes of acute pancreatitis, which is known to further lead to the development of chronic pancreatitis [3, 20].

Therefore, further research with the definition of parallels between the pathogenetic links of the metabolic syndrome, chronic pancreatitis and gallbladder cholesterosis is promising.

Regarding the morphological aspects of the study, we could not prove significant differences between the studied groups.

The formation of pseudo-adenomatous structures in the muscular layer, in our opinion, is associated with an excessive increase in the tortuosity of ducts of Luschka, which is possible with sclerosis, atrophy and a decrease in the thickness of the gallbladder wall. Ducts of Luschka are not known to be associated with the secretion of bile by the bile ducts, they are directed towards the bile ducts of the liver and flow directly into them. The formation of such adenomatous structures is a clear morphological sign of bile flow blockade in them. Unfortunately, the functional purpose of ducts of Luschka, which connect with the bile ducts of the liver in the periodical literature is insufficiently described [6, 24]: these are alternative structures of outflow or inflow of bile into the gallbladder.

In the pathogenesis of atrophy and sclerosis of the mucous membrane and other layers of the gallbladder in chronic calculous cholecystitis and cholesterosis, most authors attach importance to the violation of lipid metabolism and inflammation [8, 12]. We agree with this opinion, but pay attention to changes in blood vessels of all layers of the gallbladder wall due to selective and uneven cholesterosis - ie the accumulation of bile pigments in endothelial cells and pericytes of both large vessels and vessels of the microcirculatory tract. Due to the above changes in blood vessels, there is a redistribution of blood in the microcirculatory tract, arteries and veins. The latter are sometimes significantly expanded with signs of stasis. Dystrophic changes in the endothelium, in our opinion, contribute not only to the development of uneven plethora of stagnant nature with significant edema, but also hemorrhage, which in turn leads to the progression of sclerotic processes.

In all cases of gallstone disease in combination with cholesterosis, histological examination of the gallbladder wall revealed pronounced dystrophic and sclerotic processes that cause deformation of folds, atrophy of anastomoses, significant expansion of crypts and intercryptal canals (tunnels) with a decrease in the number of and epithelial atrophy.

The above-described signs of proliferative aseptic

inflammation may be exacerbated by the development of gallbladder wall dystonia caused by peripheral nerve dystrophy, in the structure of which dust-like inclusions of yellow-brown pigment were also determined.

Such changes certainly take a long time to develop. Morphogenesis and pathogenetic mechanisms of their development in the complex can be difficult to assess. For example, in the periodical literature we did not find works that would determine the significance of impaired lymph circulation and significant edema of the gallbladder wall, which in some cases is excessive, and in others absent. It is known that the outflow of lymph in the gallbladder occurs in the same direction as the bile. Dyskinesia of the gallbladder wall certainly affects not only the evacuation of bile, but also the evacuation of lymph, the production of which is enhanced by hemodynamic disorders. Lymphostasis is known to exacerbate hypoxia and the development of sclerosis as a result of collagenization of reticular and elastic fibers [5]. This pathogenetic mechanism of progressive sclerosis, as well as the cellular synthesis of collagen fibers in the presence of inflammatory infiltration should be further investigated at the histochemical level.

According to the literature, epithelial lining of the surface of folds, crypts, their sinuses and existing tunnels between them normally provides the absorption of liquid components of bile and increase its concentration in the gallbladder, as in the bile depot [9, 32]. With the spread of desquamation of the prismatic epithelium and its atrophy, metaplasia in the multilayered squamous function of absorption and concentration of bile and regulation of its biochemistry

decreases, while suppressing the mucus-forming function of the epithelium, which aims to thin bile [2, 27]. Deficiency of this function may also play a role in changes in bile composition and the development of chronic calculous cholecystitis. This opinion is confirmed by compensatory swelling of a prismatic epithelium in separate saved sinuses of crypts. With a reduced number of crypts and their sinuses, changes in their lumen and desquamation of the epithelium, this function is suppressed. Similarly, the mucus-forming function on the surface of the folds is suppressed, especially if the surface of the villi, which is represented by xanthoma cells is not of epithelial origin.

Thus, the morphological changes of the gallbladder wall in chronic calculous cholecystitis with cholesterolosis are diverse, as well as the pathogenetic mechanisms of their development and require in-depth study to select and improve adequate treatments. In addition, the relationship between the development of metabolic syndrome and gallbladder cholesterolosis requires further careful study.

Conclusions

1. According to the morphological study in patients with metabolic syndrome and chronic pancreatitis, operated on for chronic calculous cholecystitis, along with the preservation of the sex and age structure of the contingent, there is a significantly higher incidence of gallbladder cholesterolosis.

2. Further research with the definition of parallels between the pathogenetic links of the metabolic syndrome and chronic pancreatitis and the development of gallbladder cholesterolosis is promising.

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ХОЛЕСТЕРОЗ ЖОВЧНОГО МІХУРА У ПАЦІЄНТІВ З МЕТАБОЛІЧНИМ СИНДРОМОМ ТА ХРОНІЧНИМ ПАНКРЕАТИТОМ

Петрушенко В.В., Гребенюк Д.І., Ляховченко Н.А., Горماش П.П.

Холестероз, як патологічний стан, що виникає внаслідок порушення обміну речовин, потенційно пов'язаний з метаболічним синдромом та хронічним панкреатитом. Мета дослідження - оцінити розповсюдження та вивчити морфологічні особливості холестерозу жовчного міхура у контингенті пацієнтів із метаболічним синдромом та хронічним панкреатитом. У дослідження було включено 82 пацієнти, яким була виконана холецистектомія з приводу хронічного калькульозного холециститу. Дослідну групу склали 37 пацієнтів із метаболічним синдромом та хронічним панкреатитом. В групу порівняння ввійшли 45 пацієнтів без клінічно підтвердженого метаболічного синдрому та хронічного панкреатиту. Відповідно до мети та завдань, виконували морфологічне дослідження стінки жовчного міхура. Дані, які підлягали статистичному аналізу, обробляли за допомогою пакету статистичних програм SPSS 20.0 for Windows. Гендерно-вікова структура досліджуваного контингенту не відрізнялася від описаної в науковій літературі. Морфологічна картина холестерозу не відрізнялася у досліджуваних групах. В слизовій оболонці відмічався значний поліморфізм складок (ворсин). В епітелії траплялися ознаки десквамації та регенерації. Мало місце порушення будови крипт та синусів Рокитанського-Ашоффа. В деяких криптах визначалися мікроконкременти. В слизовій оболонці виявлялася атрофія та склероз стромы ворсинок. Звертало увагу значне розширення просвіту вен у власній пластинці слизової оболонки та перимускулярному шарі стінки жовчного міхура. В просвіті кровоносних судин

виявлялися ознаки стазу. Просвіт артерій був місцями звужений через набухання та накопиченні пігменту жовто-коричневого кольору. Визначалися ознаки спазму та значного склерозу стінки. Дистрофічні зміни в ендотелії сприяють не тільки розвитку нерівномірного повнокрі'я застійного характеру при значному набряку, а і крововиливам. Характерним був значний набряк всіх шарів, що спричиняє їх дезінтеграцію. Значне розширення лімфатичних судин зумовлює суттєве потовщення стінки жовчного міхура. Місцями спостерігається нерівномірна гіпертрофія м'язового шару. Спостерігалось надмірне збільшення звивистості каналів Люшка. За даними дослідження у пацієнтів із метаболічним синдромом та хронічним панкреатитом, прооперованих з приводу хронічного калькульозного холециститу, поряд із збереженням гендерно-вікової структури контингенту, спостерігається достовірно вища частота зустрічаємості холестерозу жовчного міхура.

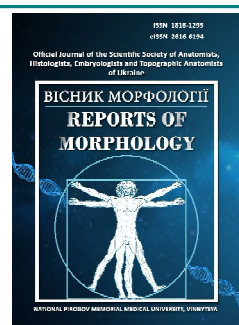
Ключові слова: холестероз жовчного міхура, метаболічний синдром, хронічний панкреатит, морфологія.



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Immunohistochemical features of expression and distribution of antibodies to thyroglobulin in the thyroid glands of newborn rats after prenatal exposure of dexamethasone

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In clinical practice, synthetic glucocorticoids, such as dexamethasone, are used to accelerate fetal maturation in pregnant women at risk of preterm birth. Increasing the concentration of cortisol in humans and other mammals often causes structural and functional changes in fetal tissues, preparing it for childbirth and extrauterine life, but they can have long-term consequences in the structural organization of organs postnatally. Despite the large number of studies on the effect of glucocorticoids on the fetus, there are almost no data on the prenatal effect of dexamethasone on the processes of synthesis and resorption of thyroglobulin by thyroid thyrocytes in the postnatal period of life. Therefore, the aim of the study was to determine the immunohistochemical features of expression and distribution of antibodies to thyroglobulin in the thyroid glands of newborn rats in normal and after prenatal exposure to dexamethasone. The study material was the thyroid gland of Wistar rats aged 1 to 7 days of postnatal development (54 animals): I group - intact animals (norm); II group - control, animals which were injected with 0.9% NaCl solution at a dose of 0.05 ml to each fetus on the 18th day of dated pregnancy; III group - experimental animals, which were administered a solution of dexamethasone at a dilution of 1:40 at a dose of 0.05 ml to each fetus on the 18th day of the date of pregnancy operatively during laparotomy, by intrauterine, transdermal subcutaneous injection into the interscapular area (Ukrainian patent №112288). Thyroglobulin Antibody (2H11) monoclonal antibodies: sc-51708 from Santa Cruz Biotechnology, Inc. were used for immunohistochemical study. Photo documentation of the studied objects was performed using a "Primo Star" microscope (Carl Zeiss, Germany) using an AxioCam camera using the Zeiss Zen program (2011). Analysis of micropreparations of thyroid glands of intact and control rats showed invariance of thyroglobulin synthesis and its accumulation, which was expressed by sufficient immunohistochemical expression of antibodies to thyroglobulin (TgAb⁺). Prenatal administration of dexamethasone leads to intensification of the processes of morphological development of hormone-producing structures (follicles and follicular epithelium), production, resorption and iodination of thyroglobulin. This is evidenced by immunohistochemical studies found in 1-3 days of the neonatal period. It should be noted that on the 7th day of life the newborn was found intense changes in the structure of the parenchyma of the thyroid gland of animals of the experimental group: increased relative percentage of follicle cavity due to increased number of large and medium, some follicles had no resorption vacuoles which was accompanied by a slowdown in the excretion of hormones into the bloodstream and led to overstretching of the follicles and, as a consequence, to the flattening of the thyroid epithelium. Intrauterine administration of dexamethasone leads to prenatal acceleration of structure formation, folliculogenesis and enhancement of hormone-producing function, which is confirmed by the peculiarities of immunohistochemical expression of TgAb. By the end of the neonatal period in rats prenatally exposed to dexamethasone, the thyroid gland is depleted of compensatory-reactive internal reserves, which is morphologically and immunohistochemically manifested by signs of hypofunction and hypertrophy. Thus, detected in the thyroid glands of animals prenatally exposed to dexamethasone,

aberration of cytoplasmic expression of TgAb⁺, intensification of colloidal expression of TgAb⁺, flattening of thyroid epithelium, and the absence of resorption vacuoles are signs of impaired hormone-forming function, which is the morphological basis for the development of hypofunctional states and requires further study.

Keywords: *thyroid gland, immunohistochemistry, thyrocyte, thyroglobulin, dexamethasone.*

Introduction

Synthetic glucocorticoids, such as dexamethasone, are used in clinical practice to accelerate fetal maturation in pregnant women at risk of preterm birth [1, 9, 18]. Antenatally, glucocorticoids mimic the effect of endogenous elevation of cortisol in blood plasma, which is usually observed in the fetus in the period close to delivery [1, 6]. In humans and other mammalian species, the surge of cortisol in the body causes structural and functional changes in the tissues of the fetus, preparing it for childbirth and extrauterine life [3, 12].

Scientists [2, 13, 19] experimentally prove that as a result of the influence of glucocorticoids on the body of animals in the postnatal ontogenesis of different age periods in the thyroid gland identified morphological signs that indicate a decrease in its functional activity. It is established that postnatal use of glucocorticoids leads to changes in the structure of the thyroid gland of rats of different ages, which are observed at all levels of its structural organization. The clinical manifestation of these changes depends on the age of the animals, the type and duration of exposure to drugs (hydrocortisone or dexamethasone). At the end of the readaptation period, the restoration of the morphofunctional state of the thyroid gland is realized more effectively in adult rats [11, 19]. Glucocorticoids affect fetal growth and tissue formation, as they are key endogenous factors that determine fetal cell maturation [10, 20, 21]. Most scientific papers on the prenatal action of exogenous glucocorticoids [2, 12, 16] have a physiological and pathophysiological direction without morphological basis of the data.

Unfortunately, despite the large number of studies on the effects of glucocorticoids on the fetus, there is almost no data on the prenatal effect of dexamethasone on the synthesis and resorption of thyroglobulin by thyroid thyrocytes in the postnatal period.

The aim of the study was to determine the immunohistochemical features of the expression and distribution of antibodies to thyroglobulin in the thyroid glands of newborn rats in normal and after prenatal exposure to dexamethasone.

Materials and methods

The study material was the thyroid glands of Wistar rats aged 1 to 7 days of postnatal development (54 animals), 6 animals in each group. Three groups of animals on the 1st, 3rd, and 7th days of the postnatal period of life were studied: I group - intact animals (norm); II group - control, animals which were injected with 0.9% NaCl solution on

the 18th day of the dated pregnancy; III group - experimental animals, which were injected with a solution of dexamethasone at a dilution of 1:40 on the 18th day of dated pregnancy (Ukrainian patent №112288). Dexamethasone and 0.9% NaCl solution were administered surgically to the fetus during laparotomy, by intrauterine, transdermal subcutaneous injection into the interscapular area at a dose of 0.05 ml.

The keeping and care of animals and all other manipulations were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), the Helsinki Declaration of the General Assembly of the World Medical Association (2000), "General Ethical Principles of Animal Experiments", adopted by the First National Congress on Bioethics (Kyiv, 2001), the Law of Ukraine "On Protection of Animals from Cruelty" (dated 21.02.2006 №3447-IV, edition dated 09.12.2015, reason 766- 19).

The thyroid complex with the tracheal area was fixed in a 10% solution of neutral buffered formalin during the day. The objects were filled into paraffin blocks by the conventional method. Histological sections 3-5 μm thick were stained with hematoxylin and eosin for viewing light microscopy.

Immunohistochemical examination was performed according to the Protocol recommended for a particular antibody of the manufacturer. Thyroglobulin Antibody (2H11) monoclonal antibodies: sc-51708 from Santa Cruz Biotechnology, Inc. were used to evaluate the morphological criteria of synthetic activity in the thyroid gland. using the method of indirect staining with immunoperoxidase using conjugated HRP mouse IgG-binding proteins, m-IgGκ BP-HRP, followed by incubation in a substrate of peroxidase and a mixture of chromogen DAB-3-diaminobenzidine tetrachloride, by staining the nuclei with Mayer's Hematoxylin Solution, dehydration, enlightenment and imprisonment in balm.

The result was regarded as positive in the precipitation of chromogen salts in the form of a specific reaction (nuclear, cytoplasmic reaction depending on the location of the antigen). The intensity of benzidine label deposition was evaluated by photographic digital morphometry using the Image J program in each case in 5 standardized fields of view of a microscope with a magnification of 400 (lens x40, eyepiece x10), where the intensity of marker expression was determined and quantified in conventional units of optical density negative reaction - 0-20; low level of

expression - 21-50; moderate level of expression - 51-100; high level of expression - more than 100.

In order to control the method, a series of studies was conducted using positive and negative samples, which served as standards.

Photo documentation of the studied objects was performed using a Primo Star microscope (Carl Zeiss, Germany) using an AxioCam camera, using the Zeiss Zen program (2011).

Results

Examination microscopy of serial histological sections of the thyroid gland of intact and control groups of 1-3 days of life revealed that the parenchyma of the organ was represented by follicles, mainly colloidal type of secretion and interfollicular thyrocytes. The cavity in the center of most follicles was not visualized, but single hollow follicles of colloidal type of small and medium diameters were chaotically located subcapsularly in the peripheral zone of the organ. At the same time, in the experimental group animals that received dexamethasone prenatally, the parenchyma of the gland looked more developed follicularly, which was expressed in the presence of hollow follicles with oxyphilically stained colloid with marginal vacuolation on the periphery of the lobe. The size of the thyroid follicles of the animals of the experimental group was significantly larger (1.6 times) compared with the control and intact groups, similar parameters of which did not differ significantly. The expression of antibodies to thyroglobulin (TgAb) in the thyroid gland of animals of the intact and control groups is moderate and is visualized in the cytoplasm of thyrocytes, in hollow follicles - in the colloid.

In the thyroid glands of animals of the experimental group, TgAb expression in the colloid of hollow follicles is uneven and has a foamy appearance due to the large

number of resorption vacuoles, while in most follicular thyrocytes cytoplasmic expression of TgAb was not detected immunohistochemically.

On the third day of postnatal life in the thyroid gland of animals of groups I and II, mostly single capsular follicles with colloidal type of secretion appear subcapsularly (Fig. 1A). Part of the spherical follicles was irregularly shaped. Cubic follicular thyrocytes lie on the basement membrane, tightly connected. There is a moderate, sometimes intense cytoplasmic TgAb expression of follicular thyrocytes (Fig. 1A). Immunohistochemically in the cavity of the follicles there is thyroglobulin with intense parietal expression and moderate central. Small marginal vacuoles of colloid resorption are partially visualized in single follicles of medium diameter. In the thyroid gland of experimental animals prenatally exposed to dexamethasone, hollow colloid-type follicles were distributed throughout the body. The follicles were mostly of medium diameter, irregular in shape and localized under the capsule. The intensity of TgAb expression is uneven throughout the follicle cavity, especially parietal in the area of resorption vacuoles. The cytoplasmic TgAb reaction is weak, visualized in single follicular thyrocytes in the apical part of the cells.

On the seventh day of life at the end of the neonatal period morphologically, the parenchyma of the thyroid gland had the following characteristics: the follicle wall was formed by prismatic and cubic thyrocytes located on the basement membrane, thyrocyte nuclei are rounded, large, well visualized nucleoli. A colloid secreted by thyrocytes and fills the follicle cavity moderately with TgAb+, mainly with parietal vacuolation (see Fig. 1A). On the 7th day of the postnatal period of ontogenesis there are intense changes in the morphological structure of the thyroid glands of animals of group III: the number of follicles of large and medium diameter increases, accompanied by a decrease

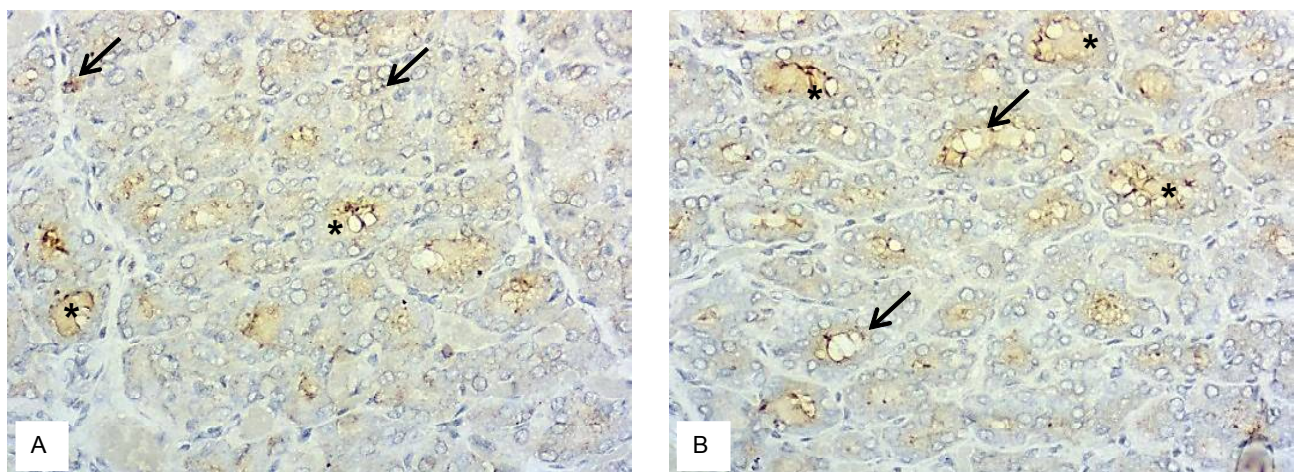


Fig. 1. Immunohistochemical expression of antibodies to thyroglobulin in the peripheral part of the thyroid gland of newborn Wistar rats on the third day of life. x400. A - thyroid gland of the control group rats; weak cytoplasmic expression of TgAb⁺ in thyrocytes of colloid-type thyroid follicles is visualized (indicated by arrows); asterisks indicate the expression of thyroglobulin colloid of the follicular cavity. B - thyroid gland of animals of the experimental group, which were prenatally administered dexamethasone; arrows indicate vacuoles of colloid resorption (TgAb⁻); asterisks indicate TgAb⁺ follicles with intense colloidal expression.

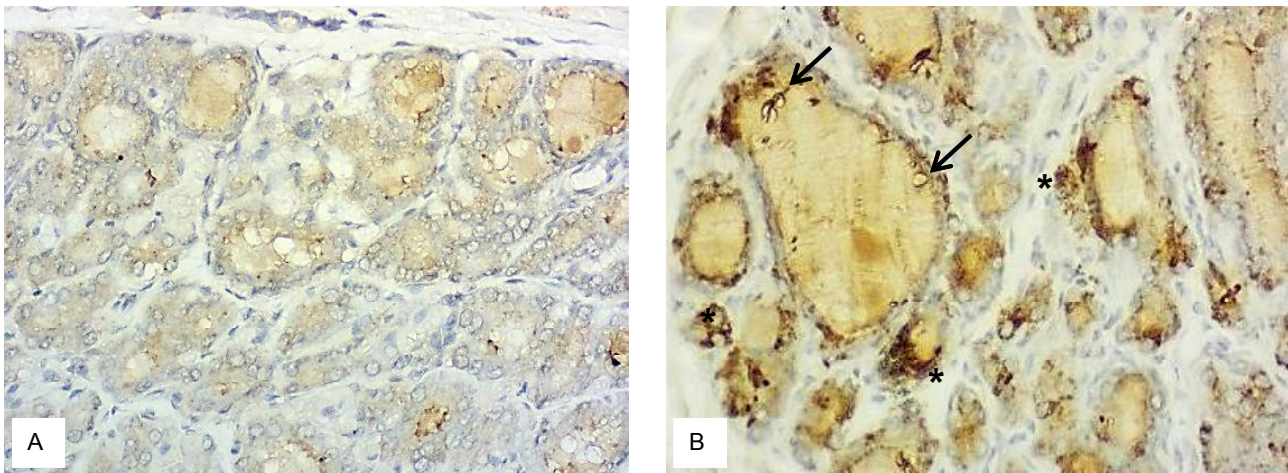


Fig. 2. Immunohistochemical expression of antibodies to thyroglobulin in the peripheral part of the thyroid gland of newborn Wistar rats on the seventh day of life. x400. A - thyroid gland of the control group rats; low cytoplasmic and moderate colloidal expression of TgAb⁺ is visualized. B - thyroid gland of animals of the experimental group, which were prenatally administered dexamethasone; intensive cytoplasmic and moderately intense colloidal TgAb⁺ expression; arrows indicate vacuoles of resorption of TgAb⁺ colloid; asterisks indicate thyrocytes with intense cytoplasmic expression of TgAb⁺.

in thyroid epithelium and an increase in colloid area. Parietal vacuolation in such follicles is absent or single vacuoles are found (Fig. 2B). There are follicles of different shapes: round, elongated, polygonal, which are mosaically distributed over the entire area of the gland.

Discussion

Analysis of thyroid micropreparations of intact and control rats showed the normality of the vector of thyroglobulin synthesis and its accumulation, which is expressed by the immunohistochemical expression of antibodies to thyroglobulin (TgAb⁺).

During the neonatal period in rats normally continues morphological development and maturation of hormone-producing structures. This process dynamically and evenly covers all parts, both intracellular and extracellular. Because thyroid hormones promote the development of other organs and structures of the body, they are intensively realized by the body on the way of adaptation of the body to extrauterine life and the development of the body as a whole. This is confirmed by the available data on the expression of antibodies to thyroglobulin and vacuolization of colloid, and similar data were obtained by scientists in control rats in an experimental study of the effects of chronic stress on morphological transformations in thyroid glands at an early age [17].

Prenatal administration of dexamethasone leads to intensification of the processes of morphological development of hormone-producing structures (follicles and follicular epithelium), production, resorption and iodination of thyroglobulin. This trend is confirmed in their studies by other scientists, who experimentally found that in fetuses of sheep induced by maternal dexamethasone, there was an increase in circulating thyroid hormone - triiodothyronine (T3) [8]. They proved that maternal

dexamethasone treatment largely mimics the effects of endogenous glucocorticoids on thyroid hormone metabolism in the near future and can promote fetal maturation by stimulating thyroid hormone activity *in utero*. This is also evidenced by our data from immunohistochemical studies, found on 1-3 day of the neonatal period. But at the end of the neonatal period (on the 7th day of life) intense transformations in the structures of the parenchyma of the thyroid glands of animals of the experimental group were revealed: the relative percentage of the area of the follicle cavity increases due to an increase in the number of large and medium follicles, in some follicles resorption vacuoles disappear, which is accompanied by a slowdown in the excretion of hormones into the bloodstream and leads to overstretching of the follicles and, as a consequence, to the flattening of the thyroid epithelium. Such morphological changes are explained and correlated with the ultrastructural features of thyrocytes studied by electron microscopic examination after postnatal administration of prednisone [5], manifested in the accumulation of colloidal droplets in the cytoplasm of follicles and proves the fact that prednisone can reduce the basal secretion of thyroid hormones by inhibiting lysosomal hydrolysis of colloid in follicular cells. That is, the consequence of severe exogenous or endogenous hypercorticism is moderate hypothyroidism [5, 7, 15, 22].

Stagnation of secretion in the cavity of the follicles leads to stretching of its walls, causing signs of hypertrophy. Aberrant cytoplasmic expression of TgAb⁺ is a sign of impaired hormone-forming function of thyrocytes, which is the morphological basis for the development of hypofunctional states, which explains the data obtained by scientists [1, 16, 19]. In the study of the effect of elevated concentrations of endogenous glucocorticoids in chronic stress on the thyroid gland at an early age, it was noted that

the greatest sensitivity to follicular compartment was found during breastfeeding, which is characterized by structural changes in it, indicating inhibition of its activity [4, 14]. It should be noted that a number of scientists in their immunohistochemical studies also note that under chronic stress, antibodies to thyroglobulin intensely stain the cytoplasm of follicular thyrocytes and are poorly uniformly expressed by colloid.

Obtained by us data that after prenatal action of dexamethasone at the end of the neonatal period in the thyroid gland are formed immunomorphological features of the hypofunctional picture, the vector of morphological changes correlates with the data of other scientists [7, 8, 22]. These studies showed that during the postnatal period due to the effects of glucocorticoids on animals of different ages, young animals were more sensitive, and after the introduction of dexamethasone lactating females in the offspring were changes in the architecture and functional activity of the thyroid gland. Thus, regardless of the route of administration, dexamethasone can cause in the thyroid glands of organisms that grow and form, such morphological signs that indicate a decrease in the functional activity of the thyroid gland, namely: reduced organometric parameters, delayed differentiation of parenchyma into lobules, increased size, flattening of the follicular epithelium with a decrease in its height,

compaction of the colloid in combination with a decrease in the size of the nuclei of thyrocytes.

The question of the occurrence in the thyroid glands of animals prenatally exposed to dexamethasone, signs of violation of the hormone-forming function of thyrocytes needs further study.

Conclusions

1. Intrauterine administration of dexamethasone leads to prenatal acceleration of structure formation, folliculogenesis and enhancement of hormone-producing function, which is confirmed by the peculiarities of immunohistochemical expression of TgAb.

2. By the end of the neonatal period in rats prenatally exposed to dexamethasone, the thyroid gland is depleted of compensatory-reactive intraorgan reserves, which is morphologically and immunohistochemically manifested by signs of hypofunction and hypertrophy.

3. Detected in the thyroid glands of animals prenatally exposed to dexamethasone, aberrations of cytoplasmic expression of TgAb⁺, intensification of colloidal expression of TgAb⁺, flattening of thyroid epithelium and practically no resorption vacuoles are signs of violation of hormone-forming function of thyrocytes, which is the morphological basis for the development of hypofunctional states.

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ІМУНОГІСТОХІМІЧНІ ОСОБЛИВОСТІ ЕКСПРЕСІЇ ТА РОЗПОДІЛУ АНТИТІЛ ДО ТИРОГЛОБУЛІНУ В ЩИТОПОДІБНИХ ЗАЛОЗАХ НОВОНАРОДЖЕНИХ ЩУРІВ ПІСЛЯ ПРЕНАТАЛЬНОГО ВПЛИВУ ДЕКСАМЕТАЗОНУ

Федосєєва О.В., Потоцька О.І.

У клінічній практиці синтетичні глюкокортикоїди, такі як дексаметазон, використовують для прискорення дозрівання плода у вагітних жінок з ризиком передчасних пологів. Підвищення концентрації кортизолу в організмі людини та інших видів ссавців нерідко викликає структурні та функціональні зміни в тканинах плода, готуючи його до пологів та позаутробного життя, але вони можуть мати віддалені наслідки в структурній організації органів постнатально. Попри велику кількість проведених досліджень щодо впливу глюкокортикоїдів на плід майже відсутні дані з пренатального впливу дексаметазону на процеси синтезу та резорбції тироглобуліну тироцитами щитоподібної залози у постнатальному періоді життя. Тому метою дослідження було визначення імуногістохімічних особливостей експресії та розподілу антитіл до тироглобуліну в щитоподібних залозах новонароджених щурів в нормі та після пренатального впливу дексаметазону. Матеріалом дослідження були щитоподібні залози щурів лінії Вістар у віці від 1 до 7 доби постнатального розвитку (54 тварини): I гр. - інтактні тварини (норма); II гр. - контрольна, тваринам котрої вводили 0,9% розчин NaCl в дозі 0,05 мл кожному плоду на 18 добу датованої вагітності; III - експериментальні тварини, яким вводили розчин дексаметазону у розведенні 1:40 в дозі 0,05 мл кожному плоду на 18 добу датованої вагітності оперативно під час лапаротомії, шляхом кризьматкової, кризьоболонкової підшкірної ін'єкції у міжлопаткову ділянку (патент України №112288). Для імуногістохімічного дослідження використовували моноклональні антитіла Thyroglobulin Antibody (2H11): sc-51708 фірми Santa Cruz Biotechnology, Inc. Фотодокументацію досліджуваних об'єктів виконано з використанням мікроскопу "Primo Star" (Carl Zeiss, Німеччина) з використанням камери АхіоСат за допомогою програми Zeiss Zen (2011). Аналіз мікропрепаратів щитоподібних залоз щурів інтактної та контрольної групи показав незмінність синтезу тироглобуліну та його акумуляції, що виражалось достатньою імуногістохімічною експресією антитіл до тироглобуліну (TgAb⁺). Пренатальне введення дексаметазону призводить до інтенсифікації процесів морфологічного розвитку гормон-продукуючих структур (фолікулів та фолікулярного епітелію), продукції, резорбції та йодиназації тироглобуліну. Про це свідчать дані імуногістохімічного дослідження, виявлені у 1-3 добу періоду новонародженості. Слід відмітити, що на 7 добу життя новонародженого були виявлені інтенсивні перетворення у структурі паренхіми щитоподібної залози тварин експериментальної групи, а саме: збільшувався відносний відсоток площі порожнини фолікулів за рахунок збільшення кількості великих та середніх, у частини фолікулів не було резорбційних вакуолей, що супроводжувалося уповільненням введення гормонів до кровотоку й призводило до перерозтягнення фолікулів і, як наслідок, до сплюснення тиреоїдного епітелію. Внутрішньоплідне введення дексаметазону призводить до пренатального прискорення структуроутворення, фолікулогенезу та підсилення гормон-продукуючої функції, що підтверджується особливостями імуногістохімічної експресії TgAb. До завершення періоду новонародженості у щурів, пренатально експонованих дексаметазоном, у щитоподібній залозі виснажуються компенсаторно-реактивні внутрішньоорганні резерви, що морфологічно та імуногістохімічно проявляється ознаками гіпофункції та гіпертрофії. Таким чином, виявлені у щитоподібних залозах тварин, пренатально експонованих дексаметазоном, аберантність цитоплазматичної експресії TgAb⁺, інтенсифікація колоїдної експресії TgAb⁺, сплюснення тиреоїдного епітелію та відсутність вакуолей резорбції є ознаками порушення гормон-утворюючої функції тироцитів, що є морфологічним підґрунтям до розвитку гіпофункціональних станів, що потребує подальшого вивчення.

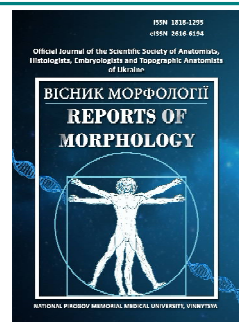
Ключові слова: щитоподібна залоза, імуногістохімія, тироцит, тироглобулін, дексаметазон.



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INDICATORS OF CARDIOVASCULAR SYSTEM IN PERSONS OF MATURE AGE DEPENDING ON A BODY CONSTITUTION UNDER ADVERSE ECOLOGICAL CONDITIONS

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Human health and its optimal functional state depend on the efficiency of the body's regulatory systems that respond to changes in a complex of exogenous and endogenous factors, ensuring timely adaptation to changing living conditions. However, natural factors, which largely determined the direction of evolution of the human body, are increasingly receding into the background, giving way to anthropogenic factors, including working conditions. That is why the aim of the scientific article was to study the state of the cardio-respiratory system in workers who are involved in various production processes, depending on their body constitution. The study of central and cerebral hemodynamics was conducted on men aged 21-35 years (90 people in total), which were divided into 3 groups, 30 persons in each: involved in industrial production, agricultural workers and residents of relatively environmentally friendly areas of Volyn region. At the first stage of the study, absolute anthropometric indicators (age, height, weight, chest circumference) were measured and the integrated indicator (Pignet index) was calculated. The next stage of the study involved functional diagnostics of the cardiovascular system of the subjects using functional ECG techniques, Kubicek rheography and rheoencephalography. Registration and analysis of relevant indicators was carried out using a set of hardware and software survey methods "Askold" (Kyiv, 1997). Statistical analysis of intergroup differences was performed using Student's t-test and Man-Whitney U-test, the level of relationship between the individual parameters was assessed by the Pearson method. It is proved that the anthropometric indicators of persons exposed to chronic exposure to harmful production factors do not differ in the group of persons involved in industrial production and in agriculture. It is shown that in subjects who are exposed to negative factors of the production environment for a long time (both during the production of agricultural products and in an industrial enterprise) there are compensatory changes in central hemodynamics (stroke volume and myocardial capacity) compared with the control group persons from ecologically clean areas of Volyn region. Employees of the industrial enterprise showed signs of deterioration of the amplitude-time characteristics of cerebral blood flow in both hemispheres, as evidenced by indicators of rapid filling time, rheographic coefficient and average blood flow velocity. These parameters differ from similar parameters of agricultural workers and residents of relatively environmentally friendly regions. Thus, the identified features of the indicators in the groups of subjects exposed to chronic exposure to negative factors of the production environment, indicate the functional nature of the identified changes that are compensatory in nature.

Keywords: factors of the production environment, central hemodynamics, cerebral hemodynamics, ECG, anthropometric indicators.

Introduction

The role of environmental factors complex in the system of formation and modification of regulatory reactions of the organism is generally accepted, but the constant

development of technologies and materials significantly affects the anatomical and physiological features of the human body [3, 9, 10, 14]. The widest range of such effects

is found in industrial production, which poses a very important task - monitoring and timely response to the emergence of "new" harmful factors of the production environment and analysis of their possible combined impact on human health [1, 11, 15, 22, 24].

It should be noted that, on the other hand, the peculiarities of the response of body systems to the action of environmental factors are determined by their own (individual) predictors. Factors that modify the impact of negative environmental factors are age, sex, health status, diet, stress resistance, the constitution of the worker's body and others [15, 16, 21, 23].

Thus, a comprehensive analysis of the studied problem on the one hand should include the study of the peculiarities of working conditions and, on the other hand, the characteristics of the workers involved in it [11, 13, 17]. That is why *the purpose* of scientific research was to study the state of the cardio-respiratory system in workers who are involved in various production processes, depending on their body constitution.

Materials and methods

Studies of central and cerebral hemodynamics were performed on men aged 21-35 years. The total number of subjects was 90 people, and, according to the purpose of the work, they were divided into three groups 30 people each, namely: control - the population living in a relatively clean ecological zone (Kivertsi region); agrarian sector - residents of agrarian districts of Volyn region, who are engaged in agriculture and are in constant contact with fertilizers and pesticides (Ivanychi and Lokachi); industry - subjects who work at industrial enterprises in the city of Lutsk. All subjects were informed about the features of the experiment and gave their consent to its conduct and processing of the results.

At the first stage of the study we performed anthropometric measurements of height, body weight and chest girth, which were performed according to the method of Bunak (1931) [7] and calculated the Pignet index (PI), which characterizes the proportionality of development and physique [18, 21]:

$$IP = L - (M + O),$$

where L - height, cm;

M - mass, kg;

O - chest circumference, cm.

To determine the type of body constitution used the classification of V.M. Chernorutsky (1927) which provides for the allocation of three categories: hyposthenics (PI>30 conventional units), normosthenics (PI 1-30 conventional units) and hypersthenics (PI<10 conventional units) [18].

The next stage of the study involved functional diagnostics of the cardiovascular system of the subjects using the ECG method, Kubicek rheography and rheoencephalography. The registration and analysis of the relevant indicators was carried out with the help of the "Askold" software and hardware complex (Kyiv, 1997).

The obtained data were processed using parametric and nonparametric methods: Student's criterion (t) when comparing averages and Mana-Whitney (U) when comparing medians. Correlation analysis of the obtained data was performed according to Pearson's test. SPSS 23.0 software was used for statistical analysis of the obtained results.

Results

Anthropometric indicators of workers involved in industrial and agricultural production and exposed to the constant influence of negative environmental factors, generally do not differ between the studied groups. However, the weight of the studied control group was significantly ($p < 0.05$) higher than in both groups involved in production. The body weight of workers in the agro-industrial complex was significantly lower than the body weight of the control group, and a similar figure for employees of industrial enterprises (Table 1).

Significant differences between the comparison groups according to the Pignet index were noted only when comparing groups from ecologically clean areas of Volyn region and agricultural workers, and higher values were noted in the latter.

The use of the Pignet index made it possible to establish certain features of the ratio of different body types of the studied contingent. In particular, it was found that among the three studied groups the predominant is the normosthenic type of physique, which was found in half of the subjects of the control group and in employees of industrial enterprises, as well as in 71% of workers in the agro-industrial complex. It should be noted that in the persons involved in agro-industrial enterprises, no persons with hypertensive body type were found, while in both other groups their number was 26% and 30%.

Modern scientific research aims to conduct a comprehensive analysis of the impact of environmental factors on the human body due to their constant deterioration. Functional reserves of the human body are increasingly strained, and regulatory systems that maintain homeostasis, show signs of dysfunction, accompanied by

Table 1. Anthropometric indicators of persons studied groups, selected taking into account complex of environmental factors ($M \pm \sigma$).

Indexes	Control group	Industry	Agricultural sector
Height, cm	178,3±5,2	179,3±7,6	177,9±4,8
Weight, kg	76,65±6,12	73,13±10,11*	67,08±5,60***
Chest circumference, cm	85,10±8,65	83,91±9,84	82,75±4,33*
Age, years	26,40±3,66	26,61±4,14	25,63±3,85
Pignet index	16,55±11,02	22,26±14,54	28,08±8,72*

Notes: * - statistically significant difference of indicators compared with the control group; ** - statistically significant difference between the groups of persons involved in agricultural production and industry.

depletion of adaptive reserves and can lead to the development of pathological conditions [2, 16]. An important aspect of this problem is the mandatory consideration of the constitutional features of man, because to a large extent they determine the "initial" state of the adaptive capabilities of the human body [21].

Our analysis of the main intervals according to the ECG at rest showed that the RR interval in the subjects involved in agricultural production was statistically significantly ($p < 0.05$) greater than in the studied workers of industrial enterprises in persons with hyposthenics body type (0.97 ± 0.28 s and 0.72 ± 0.15 s, respectively), while in normosthenics there is an inverse relationship: higher values are observed in persons involved in industrial production (0.86 ± 0.15 s and 0.97 ± 0.12 s, respectively). At the same time, there are no significant intergroup differences between these groups compared to the control group.

The duration of the PQ interval in the subgroup of hyposthenics did not show a significant difference in values between the main comparison groups. In persons involved in industrial production with hypersthenic body type there is a significant ($p < 0.05$) lengthening of the interval compared with the control group. Subjects with normosthenic type of physique are characterized by a significant ($p < 0.05$) increase in the PQ interval in workers of the agro-industrial complex when comparing similar parameters of the inhabitants of relatively ecologically clean areas.

The values of the QT segment of the electrocardiogram in the studied with different ratios of harmful production factors revealed statistically significant ($p < 0.05$) differences in the groups of hyposthenics and normosthenics, selected on the basis of the Pignet index, while hypersthenics did not show significantly significant intergroup differences in QT interval (Fig. 1).

Thus, in particular, in the group of hyposthenics there is a significantly ($p < 0.05$) shorter QT interval in persons involved in industrial production, compared with workers in the agricultural sector. In normosthenics, statistically significant ($p < 0.05$) differences in the duration of the QT interval were observed when comparing the latter with the inhabitants of relatively environmentally friendly regions.

Analysis of the QRS interval showed that regardless of the type of physique, the difference in values between the comparison groups does not reach the level of statistically significant, and the values range from 0.07 to 0.09 s.

The study of central hemodynamics has revealed a number of features in people with different body types under conditions of chronic exposure to harmful production factors. The stroke volume of blood, which characterizes the strength and efficiency of heart rate, and its minute volume show statistically significant ($p < 0.05$) differences in both groups of subjects involved in production, compared with the control group, both in normosthenics and hypersthenics. In individuals with hyposthenic physique, intergroup differences were not detected by any of the

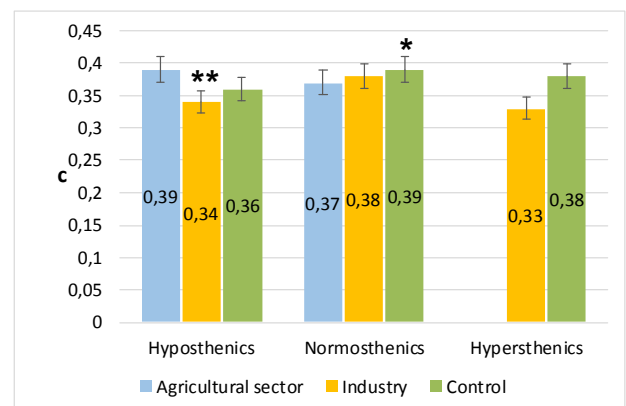


Fig. 1. Indicators of QT interval duration in subjects exposed to chronic environmental factors depending on body type. * - statistically significant difference of indicators compared with the control group; ** - statistically significant difference between the groups of persons involved in agricultural production and industry.

indicators.

The volumetric emission rate did not reveal a significant difference in values between the comparison groups on the characteristics of the environmental impact of harmful production factors, selected taking into account the Pinier index, and its values ranged from 0.21 to 0.27 ml/s.

Myocardial power values are characterized by a statistically significant ($p < 0.05$) difference between the two studied groups of persons (involved in industrial production and the agricultural sector) and residents of a relatively clean area only in the group of normosthenics. No significant intergroup differences were found in subjects with hypo- and hypersthenic body constitution.

As in the analysis of the previous indicator, the values of mean dynamic pressure and myocardial energy expenditure are characterized by the presence of significant ($p < 0.05$) differences only in the group of normosthenics. In particular, it is shown that the studied parameter is higher in the group of persons involved in the agrarian sector, compared with the control group. No statistically significant intergroup differences were found in the subjects of hypersthenic and hyposthenic body type.

Thus, the study of central blood flow and electrical activity of the myocardium when dividing the study into groups by body type made it possible to identify a significant number of differences between the studied indicators in groups with different types of production conditions: the largest number of intergroup differences is observed in hypersthenics (stroke and minute blood volumes, stroke and cardiac indices, as well as a decrease in peripheral resistance), while in other groups there are significant differences. According to the ECG data, a significant difference was found between the values of the RR interval in the subjects with hypo- and normosthenic body constitution when comparing groups from the agricultural and industrial sectors. In hypersthenics, there was a difference between the duration of the PQ interval between

the control group and workers involved in industrial production.

The next stage of the study was to determine the level of the relationship between the parameters of central hemodynamics and the Pignet index in the studied groups of individuals, selected by the characteristics of the impact of harmful factors of the working environment. The analysis showed that the highest level of correlation was observed in the control group, while the number of workers and agro-industrial complexes and the level of identified relationships were much lower. The highest values of the correlation coefficient were observed for the indicators of the minute volume of blood flow ($r=0.49$), cardiac index ($r=0.47$) and total peripheral resistance ($r=-0.41$), while the other indicators are characterized by the level of correlation in the range of 31-34%.

Under conditions of long-term exposure to harmful environmental factors, changes in blood flow are primarily found in medium and small vessels, which is reflected in reduced regional blood flow, changes in vascular tone and dysregulatory effects, which are observed by some scientists [12, 25].

The analysis of the main parameters of REG in groups selected on the basis of the Pignet index showed that in subjects with hyposthenic type there are statistically significant ($p<0.05$) higher values of pulse blood supply in both hemispheres in subjects involved in agro-industrial production, compared to with the control group and with industry workers.

In hypersthenics during the experiment there was no significant difference of Period of Pulse Blood Supply values between the comparison groups, while in normosthenics intergroup differences were found in different hemispheres: in the right marked significantly ($p<0.05$) higher values in the group of persons involved in agricultural production (0.76 ± 0.10 s) compared with the control group (0.87 ± 0.06 s), and in the left - lower ($p<0.05$) values (0.76 ± 0.10 s), compared with the group of persons from industrial enterprises (0.88 ± 0.27 s).

Analysis of the values of the time of rapid filling of the vessels of the brain, taking into account the type of physique of the subjects showed that in the group of hyposthenics there are statistically significant ($p<0.05$) lower values in both hemispheres compared with those involved in industrial (0.04 ± 0.01 s on the left and 0.05 ± 0.01 s on the right) and agricultural (0.06 ± 0.02 s on the left and 0.06 ± 0.01 s on the right) productions.

In the group with normosthenic body type, industrial persons were characterized by significantly ($p<0.05$) lower values of rapid filling time compared with the group involved in agricultural production, and with a group of residents of relatively clean areas of Volyn region (in the right hemisphere 0.03 ± 0.01 s versus 0.05 ± 0.01 s and 0.06 ± 0.01 s, respectively). In the left hemisphere, statistically significant ($p<0.05$) lower values of the fast filling time were observed in employees of industrial enterprises only in

comparison with similar indicators of the control group.

In hypersthenics, the values of the fast filling time differ ($p<0.05$) only in the left hemisphere when comparing the indicators of those studied in industrial production and the control group (0.05 ± 0.01 s and 0.08 ± 0.02 s, respectively).

The values of the maximum filling time in absolute terms were higher in the subjects involved in industrial and agricultural production. It should be noted that the level of significantly significant ($p<0.05$) they reached only in the right hemisphere when comparing the control group and employees of industrial enterprises of persons with hypersthenic body type.

The value of the latency delay time, which characterizes the peculiarities of blood flow along the main vessels of the brachiocephalic trunk, taking into account the physique of the subjects did not reveal significantly significant differences between the comparison groups. All values were distributed within 0.14-0.17 s and were within normal limits. The only intergroup difference of the indicator is observed in the right hemisphere in the group of normosthenics: significantly higher ($p<0.05$) values occur in the studied industrial enterprises, compared with the control group of individuals.

The rheographic index of the subjects was characterized by an inhomogeneous distribution of values among the comparison groups: in the group of hypersthenics involved in industrial production, there were significantly higher ($p<0.05$) values in the right hemisphere (0.09 ± 0.01 Ohm), compared with the left (0.07 ± 0.02 Ohm).

Comparison of the values in the subjects, taking into account the groups selected by the Pignet index, showed that hyposthenics in both hemispheres had a statistically significant ($p<0.05$) higher values in the group of agricultural workers, compared with industrial workers and the control group.

In normosthenics, significantly significant ($p<0.05$) differences were observed only in the right hemisphere and they are associated with statistically significantly lower values of the rheographic index in the group of industrial workers, compared with the other two comparison groups.

Subjects with a hypersthenic type of body constitution are not characterized by significantly significant intergroup differences in the values of the rheographic index throughout the study.

The average blood flow rate was statistically significantly ($p<0.05$) higher in the right hemisphere of the subjects with hyposthenic physique of persons involved in agro-industrial production compared with a similar parameter of the control group. Normosthenics have significantly lower values in the group of industrial workers compared to other groups. The left hemisphere is characterized by the presence of intergroup differences only when comparing the performance of industrial workers and the control group (significantly lower values were observed in industrial workers).

The coefficient of asymmetry of blood supply to the

vessels of the brain was within normal limits only in the control group of subjects, regardless of the type of body constitution.

Thus, the features of cerebral blood flow revealed the largest number of intergroup differences in the group of normosthenics, which was associated with the place of work of the subjects. The study found higher values of the coefficient of asymmetry of blood supply to the vessels of the brain in the group of industrial workers compared with employees of agricultural enterprises and the control group. This difference was observed in all groups, without exception, selected by body mass index.

Analysis of the correlation coefficients of the obtained parameters of cerebral blood flow with anthropometric indicators of individuals of all groups showed that the studied control groups are characterized by the highest number of high correlations between these indicators of both hemispheres. The greatest correlations were observed between the indicators of the Pignet index in the right hemisphere with the period of pulse oscillation ($r=-0.30$), the time of rapid ($r=-0.38$) and maximum ($r=-0.69$) blood filling, the time of rheowave delay ($r=-0.59$) and rheographic index ($r=-0.38$), in the left - with the values of time of rapid ($r=-0.42$) and maximum ($r=-0.32$) blood filling, time of delay of the rheowave ($r=-0.36$) and dichroic index ($r=-0.44$).

Discussion

The analysis of anthropometric data in subjects exposed to chronic exposure to a complex of harmful factors of the production environment showed that in comparison with the control group there are no differences in height, body weight, chest circumference, which indicates the homogeneity of the sample. The use of the Pignet index showed that in both groups of subjects there is no significant difference of this indicator between the comparison groups.

Systematic analysis of central and peripheral hemodynamics showed that the greatest number of differences in the values of the studied parameters are observed in workers who are negatively affected by harmful factors during production, regardless of the place of work (industry or agriculture). However, the peculiarities of indicators in these groups indicate the functional nature of the identified changes that are compensatory in nature.

The highest level of correlation of the studied indicators of the cardiovascular system according to the correlation analysis was observed in persons who do not have contact with harmful environmental factors at work, while the studied from both groups of comparisons are characterized by much lower correlations in absolute terms and their number. At the same time, the largest number of significantly high correlations was observed between the studied parameters of the circulatory system and the Pignet index, which objectifies the division of the studied by this indicator for further analysis.

It should be noted that a large number of scientists who

study the impact of exogenous factors on health and functionality of the body, say that the set of negative factors of production conditions is a fairly controlled value and the possibility of negative changes in workers can occur only in violation of technological process or accidents [11]. However, given the chronic impact of such conditions and a significant number of endogenous factors that can modify the impact of negative production factors, increasing the body's sensitivity to them, this issue is constantly discussed in scientific circles [1, 20].

In addition, the appearance of occupational diseases as the only indicator of negative changes in the human body is unacceptable, because in this case the level of "quality of life" is leveled, which is not determined only by the absence of disease [13]. Important in the context of this problem is that the functional changes caused by the studied environmental factors are often transient [5, 6, 8], or masked by the stress of adaptive capacity [4, 19], and therefore often not detected in during professional medical examinations of employees [12]. The following data, in particular, are given in the study of cerebral blood flow in persons exposed to chronic ionizing radiation in residents of radiation-contaminated regions: background REG in this contingent does not exceed the age norm, but the performance of intellectual tasks of varying complexity elasto-tonic changes of the vascular bed and a certain level of regulatory changes in the circulatory system [25].

Further research on this topic, in our opinion, should be aimed at studying the features of the cardio-respiratory system of persons affected by negative factors, taking into account the type of their physique during functional tests, because the changes we noted from the cardiovascular system are physiological in nature and in conditions of functional rest may not be fully manifested.

Conclusions

1. Anthropometric study of persons exposed to chronic exposure to harmful factors of production, showed no significant changes in both the group involved in industrial production and in agriculture.

2. According to the indicators of the Pignet index, the predominance of the normosthenic type of physique was revealed in all subjects, regardless of the peculiarities of the influence of harmful factors.

3. The study of central blood flow in subjects, taking into account the type of body constitution showed that in the group of hypersthenics who are employees of an industrial enterprise, there is an increase in stroke and minute blood volumes, as well as a decrease in peripheral resistance. In the group of normosthenics there are differences in the indicators of myocardial power and specific peripheral resistance in comparison with the control group of subjects.

4. In the group of hypostenics there was a significantly lower value of the period of pulse oscillation and rheographic index compared to other study groups. In hypersthenics patients, significant differences were

observed for indicators of rapid filling time, rheographic and dichroic indices. In normosthenics, most of the differences are observed for the parameters of the right hemisphere of persons working in industrial production,

compared with other study groups in terms of fast filling time, rheowave delay time, rheographic index and average blood flow velocity.

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ОСОБЛИВОСТІ ПОКАЗНИКІВ СЕРЦЕВО-СУДИННОЇ СИСТЕМИ В ОСІБ ЗРІЛОГО ВІКУ ЗАЛЕЖНО ВІД КОНСТИТУЦІЇ ТІЛА ЗА НЕСПРИЯТЛИВИХ ЕКОЛОГІЧНИХ УМОВ

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Здоров'я людини та її оптимальний функціональний стан залежать від ефективності роботи регуляторних систем організму, які реагують на зміни комплексу екзо- та ендогенних чинників, забезпечуючи своєчасну адаптацію до зміни умов існування. Однак, природні фактори, які значною мірою визначали напрям еволюції організму людини, на сьогодні все частіше відходять на другий план, поступаючи антропогенним чинникам, зокрема умовам праці. Саме тому метою наукової статті стало вивчення стану кардіо-респіраторної системи у працівників, які задіяні у різних виробничих процесах, залежно від їх конституції тіла. Дослідження показників центральної та мозкової гемодинаміки проводили у чоловіків віком 21-35 років (усього 90 осіб), які були поділені на 3 рівні групи: задіяні в промисловому виробництві, працівники сільськогосподарських виробництв та жителі відносно екологічно чистих районів Волинської області. На першому етапі дослідження були виміряні абсолютні антропометричні показники (зріст, маса, окружність грудної клітки) та розрахований інтегральний показник (індекс Пінье). Наступний етап дослідження передбачав проведення функціональної діагностики серцево-судинної системи досліджуваних з використанням функціональних методик ЕКГ, реографії за Кубічеком та реоенцефалографії. Реєстрацію та аналіз відповідних показників здійснювали за допомогою комплексу апаратних і програмних методів обстеження "Аскольд" (Київ, 1997). Статистичний аналіз міжгрупових відмінностей показників здійснювали за допомогою t-критерію Сьюдента та U-критерію Мана-Уїтні, рівень взаємозв'язку між окремими параметрами оцінювали за методом Пірсона. Доведено, що антропометричні показники осіб, що піддаються хронічному впливу шкідливих виробничих чинників, не відрізняються як у групі осіб, задіяних у промисловому виробництві, так і в сільському господарстві. Показано, що у досліджуваних, які тривалий час зазнають впливу негативних чинників виробничого середовища (як під час виробництва сільськогосподарської продукції, так і в умовах промислового підприємства) спостерігаються компенсаторні зміни показників центральної гемодинаміки (ударного об'єму крові та потужності міокарда) порівняно з контрольною групою осіб з екологічно чистих районів Волинської області. У працівників промислового підприємства відмічені ознаки погіршення амплітудно-часових характеристик мозкового кровотоку обох півкуль, про що свідчать показники часу швидкого наповнення, реографічного коефіцієнта та середньої швидкості кровотоку. Ці параметри відрізняються від аналогічних параметрів як працівників сільськогосподарського виробництва, так і жителів відносно екологічно чистих регіонів. Таким чином, виявлені особливості показників у групах досліджуваних, що зазнають хронічного впливу негативних факторів виробничого середовища, свідчать про функціональний характер виявлених змін, що носять компенсаторний характер.

Ключові слова: фактори виробничого середовища, центральна гемодинаміка, мозкова гемодинаміка, ЕКГ, антропометричні показники.

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For publication, scientific articles are accepted only in English only with translation on Ukrainian, which contain the following necessary elements: UDC code; title of the article (in English and Ukrainian); surname, name and patronymic of the authors (in English and Ukrainian); the official name of the organization (institution) (in English and Ukrainian); city, country (in English and Ukrainian); structured annotations (in English and Ukrainian); keywords (in English and Ukrainian); introduction; purpose; materials and methods of research; research results; discussion; conclusions; bibliographic references.

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The introduction reflects the state of research and the relevance of the problem according to the world scientific literature (at least 15 references to English articles in international journals over the past 5 years). At the end of the entry, the purpose of the article is formulated (contains no more than 2-3 sentences, in which the problem or hypothesis is addressed, which is solved by the author).

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The section should allow other researchers to perform similar studies and check the results obtained by the author. If necessary, this section may be divided into subdivisions. Depending on the research objects, the ethical principles of the European Convention for the protection of vertebrate animals must be observed; Helsinki Declaration; informed consent of the surveyed, etc. (for more details, see "Public Ethics and its Conflict"). At the end of this section, a "statistical processing of results" section is required, which specifies the program and methods for processing the results obtained by the automobile.

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In the discussion, it is necessary to summarize and analyze the results, as possible, compare them with the data of other researchers. It is necessary to highlight the novelty and possible theoretical or practical significance of the results of the research. You should not repeat the information already listed in the "Introduction" section. At the end of the discussion, a separate paragraph should reflect the prospects for using the results obtained by the author.

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5-10 sentences that summarize the work done (in the form of paragraphs or solid text).

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